

Lincoln University Digital Thesis

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.

**The effect of fish protein powder on the
physiochemical, nutritional and sensory properties
of cereal based products**

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy in Food Science

at
Lincoln University
by
Ajay Shivajirao Desai

Lincoln University
2019

Declaration

Some aspects of this thesis have been published and submitted for publication in international peer review journals and presented at conferences.

❖ Paper Published

1. Ajay Desai., Margaret A Brennan., & Charles S Brennan (2018). The effect of semolina replacement with protein powder from fish (*Pseudophycis bachus*) on the physicochemical characteristics of pasta. ***LWT-Food Science and Technology*, 89, 52-57.**
2. Ajay S. Desai., Margaret A. Brennan., & Charles S. Brennan (2018). Effect of fortification with fish (*Pseudophycis bachus*) powder on nutritional quality of durum wheat pasta. ***Foods*, 7, 62-74.**
3. Ajay S. Desai., Margaret A. Brennan., & Charles S. Brennan (2018). Influence of semolina replacement with salmon (*O. tschawytscha*) powder on the physicochemical attributes of fresh pasta. ***International Journal of Food Science and Technology*, 53 (6), 1425-1435.**
4. Ajay S. Desai., Margaret A Brennan., & Charles S Brennan (2018). Amino acid and fatty acid profile and digestible indispensable amino acid score of pasta fortified with salmon (*O. tschawytscha*) powder. ***European Food Research and Technology*, 244 (10), 1729-1739.**
5. Ajay S. Desai, Tang Beibeia, Margaret A. Brennan, Xinbo Guo, Xin-An Zeng and Charles S. Brennan (2018). Protein, amino acid, fatty acid composition, physical properties and *in vitro* starch and protein digestibility of bread fortified with salmon (*O. tschawytscha*) powder. ***Nutrients*, 10, 1923-1940.**
6. Ajay S. Desai, Margaret A. Brennan, Xinbo Guo, Xin-An Zeng and Charles S. Brennan (2019). Effect of salmon (*O. tschawytscha*) powder on starch and protein digestibility profile and antioxidant potential of semolina based pasta. ***Molecules (Accepted for publication)***

❖ Papers submitted

1. Ajay S. Desai., Margaret A. Brennan., & Charles S. Brennan. Physicochemical and nutritional characteristics of wheat bread enriched with cod fish (*Pseudophycis bachus*) powder. ***Journal of Food Biochemistry***
2. Ajay S. Desai, Margaret A. Brennan, Xinbo Guo, Xin-An Zeng and Charles S. Brennan. Effect of incorporation of fish oil and wheat gluten to wheat starch on physiochemical, structural and *in vitro* digestion properties of starch. ***Food Hydrocolloids***
3. Ajay S. Desai, Margaret A. Brennan, Xinbo Guo, Xin-An Zeng and Charles S. Brennan. Effect of incorporation of fish oil to wheat starch on pasting, textural, *in vitro* starch digestibility and structural properties of the starch. ***International Journal of Biological Macromolecules***

❖ Oral presentation and posters

1. Utilisation of fish as a protein supplement to enrich snack foods for better nutrition. A poster presentation at the **Nutrition Society of New Zealand Annual Conference, Christchurch, New Zealand in November, 2016.**
2. Effect of semolina replacement with fish powder on cooking quality, glycaemic impact and protein digestibility of pasta. An oral presentation at **67th Australasian Grain Science Conference, Christchurch, New Zealand in September, 2017.**
3. Effect of incorporating fish powder into semolina pasta on its cooking quality, *in vitro* glycaemic impact, protein digestibility and antioxidant activity. An oral presentation at **31st European Federation of Food Science and Technology (EFFoST) International Conference, Melia Sitges, Spain from 13th to 16th November, 2017.**
4. Fortifying pasta with salmon (*Oncorhynchus tshawytscha*); its effect on nutritional quality. A poster presentation at **Riddet Institute Conference, Wellington, from 10th to 12th July, 2018.**

5. Protein, amino acid, fatty acid composition and *in vitro* digestibility of bread fortified with *Oncorhynchus tshawytscha*, **Global Food Science Conference (Innovation, Communication and Collaboration)**, from 14th to 18th November, 2018 at Wuxi, China

❖ Award

1. **Food Travel Award-2017** received from Foods Journal, Switzerland for attending EFFoST international conference (www.mdpi.com/journal/foods).
2. **Overseas placements Award** for PhD students from Riddet Institute, Massey University, Palmerston North, New Zealand for perusing research work on South China University of Technology (SCUT), Guangzhou, China.

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Food Science.

The effect of fish protein powder on the physicochemical, nutritional and sensory properties of cereal based products

by

Ajay Shivajirao Desai

Starch based snack foods from wheat, corn, and rice are rich in carbohydrate and low in protein, essential amino acids and ω - fatty acids. These snacks are considered nutritionally imbalanced as a food. Consumption of highly digestible starch products may be linked to nutritional and health issues with high glycaemic index. Demand for healthier food products is increasing all over world and cereal food industry has attempted to enhance the nutritional content of products by addition of protein and lipid rich ingredients. Fish proteins are rich in all the essential amino acids (particularly methionine, lysine, histidine, threonine and valine) and long chain polyunsaturated fatty acids (LC-PUFA) (ω -3) including docosahexaenoic (DHA) and eicosapentaenoic (EPA) acid. This is in contrast to most protein from plant sources such as wheat, rice, maize, barely, soybean and pea, which lack adequate amounts of one or more essential amino acids and LC-PUFA. Cereal products can be fortified with protein and lipid rich ingredients such as fish protein concentrate (Brennan, 1992). From a nutritional point of view, fish and fishery products represent a good potential source of protein, essential amino acids and fatty acids for balanced nutrition and good health. Fish proteins have strong interactions with other proteins and high gelation ability and now a days are used in the food industry as a binder, dispersing agent and emulsifier. Cereal products can be fortified with fish proteins to reduce the glycaemic impact of such foods and provide a balanced nutritional profile for human beings as well as maintain the quality characteristics suitable for consumer acceptability. The nutritional value of such fortified foods is dependent not only on the

quantity of protein incorporated but also the quality of such proteins used, for instance their essential amino acid content and score, potential protein digestibility and digestible indispensable amino acid score (DIAAS). Fish proteins are effectively more readily digested than those of plant protein.

In this study, two different types of fish powder, cod (*Pseudophycis bachus*) and salmon (*Oncorhynchus tshawytscha*), were added into fresh pasta (cold extrusion and cooking) and bread (fermented cooking). The physical, chemical, textural, nutritional and sensory properties of these products were determined. These developed products may alter the Western consumer diet habits to fulfil the protein, essential amino acid and $\omega 6:\omega 3$ ratio though cod and salmon fish powder.

Replacement of durum wheat semolina with different levels of cod (*P. bachus*) fish powder (CFP) was undertaken to increase the physicochemical, nutritional and sensory value of pasta. The result showed that fortification with CFP increased cooking loss as well as decreased optimal cooking time, swelling index and water absorption, whilst increasing firmness and resistance to uniaxial extension of pasta. The addition of CFP increased yellowness (b^*) of the pasta compared to control sample. Furthermore, CFP enriched protein, ash and energy content of pasta samples compared to control sample. Incorporation of CFP showed a significant decrease ($P < 0.05$) in reducing sugar released during an *in vitro* digestion and standardised AUC values compared to control pasta. The potentially bio-accessible fraction of pasta enriched with 20% fish powder (FP) was characterised by a 177-191% increase in phenolic content and a 145-556% higher antiradical activity. Supplementation of fish powder also influenced protein digestibility (a reduction from 84.60% for control pasta to 80.80% for pasta with 20% fish powder). The sensory evaluation of CFP enriched pasta products was conducted using sensory panel. The result exhibited that all samples (5-15% CFP) were acceptable.

A partial substitution of durum wheat semolina with different levels of salmon (*O. tshawytscha*) fish powder (SFP) was undertaken to increase the nutritional and sensory value of pasta. The results demonstrated that pasta with SFP had increased protein (12.88%-23.40%), lipid (0.46%-7.20%), ash (0.39%-0.57%) and energy (122.26-161.08 kcal) contents as well as increased cooking time and cooking loss. The addition of SFP resulted in significantly decreased swelling, water absorption index and firmness whilst increasing resistance to uniaxial extension of pasta. Colour parameters indicated comparable brightness between the samples and higher redness values for enriched pasta. Furthermore, the starch digestibility of pasta decreased significantly as SFP levels increased with glycaemic measurements ranging between 505-382 mg/g (108 to 143% lower than control) and the values for the glycaemic area under curve (AUC) 385-261 for 5-20% of supplemented pasta, respectively. Incorporation of SFP affected the *in vitro* bioaccessibility of nutrients. The phenolic bio-accessible fraction of pasta enriched with 20% SFP was 179% (gastric) – 133% (pancreatic) increased compared to the control pasta, and the antioxidant activity was 263% (gastric)-190% (pancreatic) higher. Supplementation of SFP reduced protein digestibility (86.41% for control pasta; 81.95% for 20% SFP pasta). The sensory evaluation of SFP enriched pasta products was conducted using sensory panel. The result exhibited that all samples (5 -15% SFP) were acceptable.

The research was undertaken to enhance the protein quality of pasta with partial substitution of durum wheat semolina with SFP. The results demonstrate that fortification of pasta with SFP increased indispensable amino acid (IAA) content compared to the control. As well as decreased the n6:n3 ratio from 19:1 (the control pasta) to 5:1 to 3:1 respectively. Digestible indispensable amino acid scores (DIAAS) were calculated using published data on amino acids digestibility to evaluate the protein quality of the pasta. IAA values (mg IAA/g protein), were found to be highest in the enriched pasta with SFP (372 to 453) and lowest in the control pasta (328), all exceeded the FAO recommended daily allowance (277 mg IAA/g protein) and

contributed on average 41% to total amino acid contents. The DIAAS value in the control pasta was 36 % (lysine) and the pasta enriched with SFP containing had a DIAAS between 75 to 99 %.

The study was undertaken to evaluate physical (moisture, volume), technological (texture and colour) nutritional (amino acid content, starch and protein digestibility) and nutraceutical (total phenolic content (TPC) and antioxidant capacity) properties of bread fortified with different levels of CFP and compared with a control bread. The result showed that there was a significant increase in the bread specific volume, crumb colour, and textural properties. The nutritional quality of the bread was analysed using an *in vitro* glycaemic response and protein digestibility digestion method. The results illustrate that the incorporation of CFP into wheat flour decreased the potential glycaemic response of bread. However, this study also shows that addition of CFP increased protein content and phenolic content, antioxidant capacity. Furthermore, incorporation of CFP into bread increased the protein quality in terms of essential amino acid content and score, protein digestibility and protein digestibility corrected amino acid score (PDCAAS).

The study was conducted to investigate protein, amino acid, fatty acid composition, *in vitro* starch and protein digestibility, phenolic and antioxidant composition of bread fortified with SFP. The proximate composition in control and SFP breads were ranged between (34-31.42%) moisture, (13.91-20.04%) protein, (3.86-9.13%) fat, (2.13-2.42%) ash, (80.10-68.42) carbohydrate and (410.8-435.96 kcal) energy. The essential amino acid of control and SFP breads ranged between 261.75-306.96 mg/g protein which satisfies the score recommended by FAO/WHO/UNU (2007). The *in vitro* assay for protein digestibility, protein digestibility amino acid score, essential amino acid index, biological value and nutritional index ranged between 79.96-80.80%, 0.15-0.42%, 62.51-76.68%, 56.44-71.68%, 8.69-15.36% respectively. Control and SFP breads contained 60.31-43.60 g/100g total fatty acids (saturated fatty acids)

and 13.51-17.00 g/100g total fatty acids (polyunsaturated fatty acids) and SFP breads fulfil the $\omega 6/\omega 3$ score recommended by food authority. There was a significant effect of SFP on bread specific volume, crumb colour, and textural properties. The *in vitro* starch digestibility results illustrate that the incorporation of SFP into wheat bread decreased the potential glycaemic response of bread and increased the antioxidant capacity of bread.

The study was undertaken to examine the effect of inclusion salmon oil (SO), cod oil (CO), coconut oil (CONT) and wheat gluten (WG) to wheat starch (WS) on pasting, textural and *in-vitro* starch digestibility properties. The TCMs and BCBs sample showed better complex index (CI) as compared to WS and WS-WG and decreased with increasing carbon chain length. The inclusion of lipids to WS significantly reduced pasting and textural characteristics than that of WS alone. In TCMs and BCBs samples V type amylose-lipid complexes peak at $20^\circ 2\theta$ and greater short range molecular order were formed with fatty acids producing more crystalline structure. Similar observation was noted by FTIR, Raman, and ^{13}C NMR spectroscopy. Differential scanning calorimetry (DSC) results suggested that decreased in the gelatinisation temperature and enthalpy after addition of different oil sources to WS. The amount of reducing sugar released during starch digestion appears to be dependent on starch hydrolysis. In this study, due to amylose-lipid complex formation, *in vitro* glycaemic response decreased in TCMs (three component mixtures) and BCBs (binary component blends) samples followed by WS-WG with the ability to act as a barrier to suppress the activity of digestive enzymes to hydrolyse the starch molecules. These altered characteristics of WS might help to formulate the foods to overcome the digestibility concern in human nutrition.

Keywords: Semolina, wheat flour, cod, salmon fish powder, pasta, bread, physiochemical, technological, starch and protein digestibility, viscosity, phenolic and antioxidant content

Acknowledgements

Firstly, I would like to express my sincere gratitude to my research guide Prof. Charles S. Brennan and advisory committee member Prof. Margaret A. Brennan for the continuous support and encouragement throughout my Ph.D study and related research, for their patience, motivation, and immense knowledge from the beginning to the end of my Ph.D study. Their guidance helped me in all the time of planning of research work and writing of this thesis and research manuscripts. I could not have imagined having a better advisor and mentor for my Ph.D study. Besides my major advisor, I would like to thank the rest of my thesis advisory committee member Prof. Stephen L. On for their insightful comments and encouragement.

My sincere thanks also goes to Prof. Zheng and Prof. Xinbo Gu who provided me an opportunity to join their team as a PhD student at South China University of Technology (SCUT), Guangzhou, China, and who gave access to the laboratory and research facilities. Without their precious support it would not be possible to conduct this research.

I thank my fellow lab mates Lu Wenjun, Lu Xikun, Gao Candy, Ade Rachman and Wu, Gang for the stimulating discussions and working together before deadlines, and for all the fun we have had in the last three years. Also, I thank my friends Fatima Jamal, Lokesh, Piyush, Yadnya, Swapnil, and Rahul.

A very special gratitude goes out to all down at Lincoln University, Faculty of Agriculture and life science for helping and providing me all support for three years. Also, I am thankful to Indian Council of Agriculture Research (ICAR), New Delhi for funding my work. Last but not the least, I am grateful to my wife Reema and daughter Riddhi for moral and emotional support throughout my Ph.D study. I am also grateful to my other family members and friends (Sai, Bharat, Sandesh and Shardul) who have supported me along the way.

Table of Contents

Declaration.....	ii
Acknowledgements	x
Table of Contents	xi
List of Tables	xviii
List of Figures	xxii
Chapter 1 Introduction	1
1.1 Background.....	1
1.2 Research gap	6
1.3 Aim of research	7
1.4 Objectives.....	7
1.5 Hypothesis.....	7
1.6 Thesis structure.....	9
Chapter 2 Review of Literature.....	9
2.1 Importance of fish consumption in human diet.....	12
2.1.1 Importance of protein.....	19
2.2 Protein digestibility	23
2.3 Review of <i>in vitro</i> methods to determine protein digestibility.....	26
2.4 Importance of cereal food.....	29
2.5 Effect of combining starch, protein and lipid sources.....	34
2.6 Starch digestibility	35
2.7 <i>In vitro</i> methods to determine starch digestibility	37
2.8 Total phenolic content (TPC) and antioxidant capacity	42
2.9 <i>In vitro</i> method of TPC and antioxidant capacity	43
2.10 Inclusion of fish protein into cereal products and its effect on <i>in vitro</i> protein and starch digestion as well as antioxidant capacity and sensory evaluation.....	47
Chapter 3 Materials and methods	54
3.1 Materials.....	54
3.1.1 Fish powder preparation.....	55
3.2 Pasta and bread manufacture.....	56
3.2.1 Flour used for pasta and bread	56

3.2.2 Pasta making process.....	56
3.2.3 Bread making process	58
3.3 Chemicals, enzymes and buffers used	59
3.3.1 Chemicals and enzymes required for the protein digestibility analysis :.....	59
3.3.2 Chemical. Buffers and enzyme required for starch digestibility analysis:	60
3.3.3 Chemicals and buffers required for total phenolic content (TPC) and antioxidant analysis:.....	62
3.4 Methods	63
3.4.1 Protein content	63
3.4.2 Moisture content:	63
3.4.3 Fat:	64
3.4.4 Carbohydrate and energy content.....	64
3.4.5 <i>In vitro</i> protein digestibility.....	64
3.4.6 <i>In vitro</i> starch digestibility and glycaemic response	65
3.4.7 Amino acid analysis of pasta and bread	66
3.4.8 Digestible indispensable amino acid score (DIAAAS).....	67
3.4.9 Fatty acid analysis of pasta and bread.....	68
3.4.10 Total phenolic content (TPC) and antioxidant capacity	69
3.4.10.3 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay	69
3.4.11 Physical characteristics of pasta	70
3.4.12 Sensory analysis	72
3.4.13 Rapid visco analyser	73
3.4.14 Complex index (CI)	74
3.4.15 Scanning electron microscopy (SEM).....	74
3.4.16 Fourier transform infrared (FT-IR) spectroscopy	74
3.4.17 Laser confocal micro-Raman (LCM-Raman) spectroscopy	74
3.4.18 Differential scanning calorimeter (DSC).....	75
3.4.19 ¹³ C nuclear magnetic resonance (NMR) spectroscopy	75
3.4.20 Statistical analysis	75
Chapter 4 The effect of semolina replacement with protein powder from fish (<i>Pseudophycis bachus</i>) on the physicochemical characteristics of pasta	76
4.1 Introduction.....	77
4.2 Materials and method.....	79
4.2.1 Raw materials.....	79
4.2.2 Fish powder preparation.....	79
4.2.3 Pasta production	79
4.2.4 Proximate chemical composition analysis of pasta	79
4.2.5 Physical properties.....	79
4.2.6 Textural characteristics	79
4.2.7 Statistical analysis	79
4.3 Results and discussion.....	80
4.3.1 Chemical composition.....	80
4.3.2 Effect of fish powder inclusion on cooking loss, swelling index and water absorption index of pasta	81
4.3.3 Colour measurement of pasta	85
4.3.4 Textural measurements	87
4.3.5 Conclusions	90

Chapter 5 Effect of fortification with cod fish (<i>Pseudophycis bachus</i>) powder on nutritional quality of durum wheat pasta	91
5.1 Introduction.....	92
5.2 Materials and Methods.....	94
5.2.1 Raw materials.....	94
5.2.2 Fish powder preparation.....	94
5.2.3 Pasta preparation	94
5.2.5 Gastric digestibility determination using <i>in vitro</i> starch digestion process.....	94
5.2.6 <i>In vitro</i> protein digestibility.....	94
5.2.7 <i>In vitro</i> gastric intestinal digestion.....	94
5.2.8 Total phenolic content in pasta	94
5.2.9 Antioxidant activity of pasta (Oxygen Radical Absorbance Capacity (ORAC) Assay	95
5.2.10 Statistical analysis	95
5.3 Results and discussion.....	95
5.3.1 Amino acid profile of semolina and fish powder	95
5.3.2 <i>In Vitro</i> Predictive Glycaemic Response.....	96
5.3.3 Protein Content and <i>In Vitro</i> Protein Digestibility	99
5.4 Phenolic content and antioxidant activity	102
5.5 Conclusions.....	105
 Chapter 6 Influence of semolina replacement with salmon (<i>Oncorhynchus tshawytscha</i>) powder on the physicochemical attributes of fresh pasta	106
6.1 Introduction.....	107
6.2 Materials and methods	109
6.2.1 Raw materials.....	109
6.2.2 Salmon fish powder (SFP) preparation	109
6.2.3 Pasta production	109
6.2.4 Proximate chemical composition analysis of pasta	109
6.2.5 Physical properties.....	109
6.2.6 Colour measurements.....	110
6.2.7 Textural measurements	110
6.2.8 Moisture content	110
6.2.9 Statistical analysis	110
6.3 Results and discussion.....	110
6.3.1 Chemical composition of pasta enriched with SFP	110
6.3.2 Effect of SFP inclusion on cooking loss, swelling index and water absorption index of pasta.....	112
6.3.3 Colour measurements.....	116
6.4 Textural measurements	119
6.5 Conclusion	121
 Chapter 7 Effect of salmon (<i>O. tshawytscha</i>) powder on starch and protein digestibility profile and antioxidant potential of semolina based pasta.....	123
7.1 Introduction.....	124

7.2 Materials and methods	126
7.2.1 Raw materials.....	126
7.2.2 Salmon powder preparation.....	127
7.2.3 Pasta production.....	127
7.2.4 <i>In Vitro</i> starch digestibility and glycaemic response	127
Described in section 3.4.6.....	127
7.2.5 <i>In vitro</i> protein digestibility.....	127
7.2.6 Amino acid profile of digesta	127
7.2.7 Total phenolic content (TPC)	127
7.2.8 Antioxidant activity (Oxygen Radical Absorbance Capacity, ORAC Assay)	127
7.2.9 Sensory analysis	127
7.2.10 Statistical analysis	127
7.3 Results and discussion.....	127
7.3.1 <i>In Vitro</i> starch digestibility and glycaemic response.....	127
7.4 Protein and <i>In vitro</i> protein digestibility	132
7.5 The composition of amino acids released into intestine after <i>in vitro</i> digestion	134
7.6 Phenolic content and antioxidant activity	135
7.7 Sensory analysis of pasta products	139
7.8 Conclusion	141
Chapter 8 Amino acid and fatty acid profile and digestible indispensable amino acid score of pasta fortified with salmon (<i>O. tshawytscha</i>) powder.....	142
8.2 Materials and methods	146
8.2.1 Raw materials.....	146
8.2.2 Fish powder preparation	146
8.2.3 Pasta production.....	146
8.2.4 Chemical composition analysis of pasta	146
8.2.5 Amino acid profile of pasta enriched with SFP	146
8.2.6 Fatty acid profile of SFP added pasta.....	146
8.2.7 Calculation of digestible indispensable amino acid score (DIAAS)	146
8.2.8 Statistical analysis	146
8.3 Results and discussion.....	147
8.4 Conclusion	157
Chapter 9 Physicochemical and nutritional characteristics of wheat bread enriched with cod fish (<i>P. bachus</i>) powder	158
9.1 Introduction.....	159
9.2 Materials and methods	161
9.2.1 Raw materials.....	161
9.2.2 Fish powder preparation.....	161
9.2.3 Preparation of bread.....	161
9.2.4 Proximate chemical composition analysis of pasta	161
9.2.5 Volume, density, moisture and texture properties of bread	161
9.2.6 Colour measurements.....	161
9.2.7 Gastric digestibility using <i>In vitro</i> starch digestion process	161
9.2.8 Amino acid profile and scoring	161

9.2.9 <i>In vitro</i> protein digestibility and protein digestibility corrected amino acid score (PDCAAS)	162
9.2.10 Total phenolic content and antioxidant activity of bread	162
9.3 Results and discussion.....	162
9.3.1 Chemical composition, energy content and specific volume of bread.....	162
9.3.2 Texture and colour measurement of bread.....	164
9.3.3 Protein quality of bread	167
9.3.4 Glycaemic glucose equivalent (GGE) analysis of bread	171
9.3.5 Total phenolic content and antioxidant activity	173
9.4 Conclusion	175
Chapter 10 Protein, amino acid, fatty acid composition, physical properties and <i>in vitro</i> starch and protein digestibility of bread fortified with salmon (<i>O. tschawytscha</i>) powder	176
10.1 Introduction	177
10.2 Materials and method.....	179
10.2.1 Raw materials.....	179
10.2.2 Fish powder preparation.....	179
10.2.3 Preparation of bread.....	179
10.2.4 Proximate chemical composition analysis of SFP enriched bread.....	180
10.2.5 Volume, density, moisture and texture properties of bread.....	180
10.2.6 Colour measurements.....	180
10.2.7 <i>In vitro</i> starch digestibility of bread	180
10.2.8 Amino acid profile and scoring	180
10.2.9 <i>In vitro</i> protein digestibility, protein digestibility corrected amino acid score (PDCAAS) and nutritional index.	180
10.2.10 Fatty acid profile of bread fortified with SFP	180
10.2.11 Total phenolic content and antioxidant activity of bread	180
10.2.12 Statistical analysis	180
10.3 Results and discussion.....	180
10.3.1 Chemical composition and physical properties of bread	180
10.3.2 Texture and colour measurement of bread.....	183
10.3.3 Protein quality of bread	186
10.3.4 Fatty acid profile of bread.....	190
10.3.5 <i>In vitro</i> starch digestion analysis.....	192
10.3.5 Total phenolic content and antioxidant activity of bread	195
10.3.6 Conclusion.....	196
Chapter 11 Effect of incorporation of fish oil and wheat gluten to wheat starch on physiochemical, structural and <i>in vitro</i> digestion properties of starch.....	197
11.1 Introduction	198
11.2 Materials and methods	200
11.2.1 Raw materials.....	200
11.2.2 Wheat starch-fishoil-gluten gel preparation	200
11.2.3 Complex Index (CI)	200
11.2.4 Pasting properties	200
11.2.5 Texture and colour characteristics.....	200
11.2.6 <i>In vitro</i> starch digestibility.....	200
11.2.7 Scanning electron microscopy (SEM).....	200
11.2.8 Fourier transform infrared (FT-IR) spectroscopy	200

11.2.9 Laser confocal micro-raman (LCM-Raman) spectroscopy	200
11.2.10 Differential scanning calorimeter (DSC).....	201
11.2.11 ¹³ C nuclear magnetic response (NMR) spectroscopy	201
11.2.12 Statistical analysis	201
11.3 Results and discussion.....	201
11.3.1 Pasting, texture and colour characteristics.....	201
11.3.2 Thermal properties and complex index (CI).....	204
11.3.3 Laser confocal micro-raman (LCM-Raman) spectra of ternary complexes and its effect on short range ordered structure.....	206
11.3.4 Effect of different oil on FTIR spectra of ternary complexes	208
11.3.5 X-ray diffraction of ternary complexes	209
11.3.6 <i>In vitro</i> starch digestibility.....	211
11.3.7 Ternary complex detection using ¹³ C nuclear magnetic response (NMR) spectroscopy	212
11.3.8 Morphological properties	214
11.4 Conclusion.....	216
Chapter 12 Effect of incorporation of fish oil to wheat starch on pasting, textural, <i>in vitro</i> starch digestibility and structural properties of the starch.....	217
12.1 Introduction	218
12.2 Materials and methods	220
12.2.1 Raw materials.....	220
12.2.2 Wheat starch-fishoil (starch-lipid) gel preparation.....	220
12.2.3 Complex Index (CI)	220
12.2.4 Pasting properties	220
12.2.5 Texture and colour characteristics.....	220
12.2.6 <i>In vitro</i> starch digestibility.....	220
12.2.7 Scanning electron microscopy (SEM).....	220
12.2.8 Fourier transform infrared (FT-IR) spectroscopy	220
12.2.9 Laser confocal micro-raman (LCM-Raman) spectroscopy	220
12.2.10 Differential scanning calorimeter (DSC).....	220
12.2.11 ¹³ C nuclear magnetic response (NMR) spectroscopy	220
12.2.12 Statistical analysis	221
12.3 Results and discussion.....	221
12.3.1 Pasting, textural and colour characteristics	221
12.3.2 Complex index (CI)	224
12.3.3 Thermal properties	225
12.3.4 Laser confocal micro-raman spectroscopy	226
12.3.5 FTIR spectra of BCBs complexes	228
12.3.6 X-ray diffraction	229
12.3.7 <i>In vitro</i> starch digestibility.....	230
12.3.8 ¹³ C nuclear magnetic response.....	232
12.3.9 Morphological characteristics	233
12.4 Conclusion	234
Chapter 13.....	236
12.1 Summary	236
12.2 Discussion.....	236
12.3 Recommendation for future work	245

Appendix.....	247
A.1 Approval letter from Human Ethics Committee.....	247
A.2 Questionnaires' for sensory analysis.....	248
A.3 QR analysis data	249
 References	 253

List of Tables

Table 2.1 Quantity of EPA and DHA in selected food (g/100g)	17
Table 2.2 Recommendations for fish and long-chain ω -3 PUFA intake from different food agency. ...	18
Table 2.3 Recommended dietary protein requirements by humans of all age groups.	20
Table 2.4 Requirements of essential amino acids (EAA) (mg amino acid/kg body weight/day) by the infants, children, adolescents and adults (FAO/WHO/UNU, 2007).	21
Table 2.5 Essential amino acid contents and nutritional value of proteins from various sources (mg/g protein) (Friedman, 1996; Sosulski & Imafidon, 1990).	22
Table 2.6 Effect of different food components on <i>in vitro</i> protein digestibility.	25
Table 2.7 Methods of <i>in vitro</i> protein digestibility, PDCAAS and DIAAS.	28
Table 2.8 The effect of protein and lipid addition to cereal based products.....	31
Table 2.9 Methods of <i>in vitro</i> starch digestibility.....	38
Table 2.10 Effect of various food components on starch digestibility and glycaemic index.	38
Table 2.11 Effect of glycaemic index (GI) and human health.	41
Table 2.12 TPC and antioxidant estimation methods.	45
Table 2.13 Influence of various food components on TPC and antioxidant activity.	46
Table 3.1 Combination of semolina and fish powder used to prepare pasta.....	57
Table 3.2 Combination of wheat flour and fish powder used to prepare bread.	59
Table 4.1 Chemical composition (%) and energy value (kcal/100g) of cooked and uncooked pasta fortified with different fish powder levels.	80
Table 4.2 Physical properties of cooked pasta products enriched with fish powder.	82
Table 4.3 Colour characteristics of cooked and uncooked pasta enriched with fish powder.	85
Table 4.4 Textural properties of enriched pasta with fish powder.....	89
Table 5.1 Amino acid profile of semolina, cod fish powder and bovine serum albumin ^a	95
Table 5.2 <i>In vitro</i> starch digestibility profile of control and pasta containing fish powder.	96

Table 5.3 Protein content, <i>in vitro</i> protein digestibility and protein availability of pasta fortified with fish powder.	100
Table 5.4 Total phenolic content and antioxidant activity of fortified pasta subjected to <i>in vitro</i> digestion.....	103
Table 6.1 Chemical composition (g/100g) (dry weight basis) and energy value (kcal/100g) of cooked and uncooked pasta enriched with different salmon fish powder (SFP) levels.....	111
Table 6.2 Physical properties of cooked pasta products enriched with salmon fish powder (SFP).	112
Table 6.3 Colour characteristics of control and salmon fish powder (SFP) enriched pasta products.	117
Table 6.4 Textural properties of enriched pasta with salmon fish powder (SFP).	119
Table 7.1 Protein content, <i>In vitro</i> protein digestibility and protein availability of pasta fortified with salmon fish powder (SFP).....	132
Table 7.2 Amino acid (AAs) composition (mg/g protein) from digestibility studies in the intestinal stage at 120 min of pasta enriched with different salmon fish powder (SFP) levels and control.....	135
Table 8.1 Chemical composition (g/100g) (dry weight basis) of cooked pasta enriched with different salmon fish powder (SFP) levels.....	147
Table 8.2 Fatty acid profile (g of individual fatty acids/100g of total fatty acids) of the cooked pasta enriched with different levels of SFP.	149
Table 8.3 Amino acid (AAs) composition (mg/g protein dry weight basis) of the cooked pasta enriched with salmon fish powder (SFP) and the control pasta.	151
Table 8.4 Adult daily recommended allowances of indispensable amino acids (IAA) and their composition (mg/g of proteins) in control and the pasta enriched with (SFP).....	153
Table 8.5 Digestible IAA content (mg/g protein) for each IAA of the cooked control pasta and the pasta enriched with SFP.....	154
Table 8.6 Dietary Indispensable amino acids (IAA) reference ratio for minimal IAA and digestible indispensable amino acid score (DIAAS) of the control pasta and the pasta enriched with (SFP).	155
Table 8.7 Dietary Indispensable amino acids (IAA) reference ratio for minimal IAA and digestible indispensable amino acid score (DIAAS) of the control pasta and the pasta enriched with (SFP).	156

Table 9.1 Bread formulations, proximate compositions and physical properties of bread. A. Ingredients used in cod powder (CP) enriched bread. B. Proximate composition of bread elaborated with CP. C. Physical properties of bread made with CP.	162
Table 9.2 Technological characteristic of bread enriched with different levels of cod powder (CB). A. Texture profile analysis. Colour characteristics of crust and crumb.....	165
Table 9.3 Amino acid (AAs) composition (mg/g protein dry weight basis) of bread enriched with different levels of cod fish powder and control.....	168
Table 10.1 Bread formulations, proximate compositions and physical properties of bread. A. Ingredients used in salmon powder (SP) enriched bread. B. Proximate composition of bread elaborated with SP. Physical properties of bread made with SP.....	181
Table 10.2 Technological characteristic of bread enriched with different levels of salmon powder (SB). A. Texture profile analysis. Colour characteristics of crust and crumb.....	184
Table 10.3 Amino acid (AAs) composition (mg/g protein dry weight basis) and nutritional characterisation of wheat bread (control bread) and breads enriched with different levels of salmon fish powder. IVPD: <i>in vitro</i> protein digestibility; EAAI: Essential Amino Acid Index, BV: biological value, NI Nutritional Index.....	187
Table 10.4 Fatty acid profile (g of individual fatty acids/100g of total fatty acids) of bread enriched with different levels of salmon fish powder (SFP).	190
Table 11.1 Pasting characteristics of wheat starch and its blend with 15% cod, salmon, coconut oil and 10% wheat gluten.....	202
Table 11.2 Texture and colour characteristics of wheat starch and its blend with 15% cod, salmon, coconut oil and 10% wheat gluten.....	203
Table 11.3 Thermal properties of wheat starch and in combination with (15%) cod oil, salmon oil, coconut oil and (10%) wheat gluten.	204
Table 11.4 FWHM of the band at 480 cm ⁻¹ band and at 20 (2θ) angle determined by Raman and XRD respectively of wheat starch and in combination with 15% cod oil, salmon oil, coconut oil and (10%) wheat gluten.	206

Table 11.5 ^{13}C - chemical shifts of wheat starch and its blend with 15% cod, salmon, coconut oil and 10% wheat gluten.	214
Table 12.1 Pasting characteristics of wheat starch and its blend with 15% cod, salmon, coconut oil and wheat gluten.	223
Table 12.2 Texture and colour characteristics of wheat starch and its blend with 15% cod, salmon, coconut oil and wheat gluten.	224
Table 12.3 Thermal properties of wheat starch and in combination with (15%) cod oil, salmon oil, coconut oil and wheat gluten.	225
Table 12.4 FWHM of the band at 480 cm^{-1} band and at $20 (2\theta)$ angle determined by Raman and XRD respectively of wheat starch and in combination with 15% cod oil, salmon oil , coconut oil and wheat gluten.	227
Table 12.5 ^{13}C - chemical shifts of wheat starch and its blend with 15% cod, salmon, coconut and wheat gluten.	232

List of Figures

Figure 2.1 Importance of fish protein and lipid in human health	13
Figure 2.2 Conversion of linoleic and α -linolenic acids into their longer chain derivatives.	15
Figure 2.3 Dietary protein intake and its digestion by different enzyme in the gastrointestinal tract.	24
Figure 2.4 Bread making process	31
Figure 3.1 Experimental design	54
Figure 3.2 Preparation of Fish powder.....	55
Figure 3.3 Pasta Extruder	57
Figure 3.4 Pasta extruder top view	57
Figure 3.5 Pasta extruder parts.....	57
Figure 3.6 Pasta extruder die	57
Figure 3.7 Bread dough mixer	58
Figure 3.8 Bread mixer parts	58
Figure 3.9 Bread moulding dish	59
Figure 3.10 Oven	59
Figure 4.1 Pasta enriched with different levels of cod powder.	86
Figure 4.2 Texture analyser and probe used for pasta firmness and extensibility.	88
Figure 5.1 Amount of reducing sugar released during <i>in vitro</i> digestion. CP-control pasta, P5-P20 pasta fortified with 5-20% fish powder, respectively.	98
Figure 5.2 The pH vs time curves obtained by pasta made with different concentration of fish powder incubated with multi-enzymes (trypsin, chymotrypsin and protease).	101
Figure 6.1 Pasta enriched different levels (5, 10, 15 and 20 %) of salmon powder.	116
Figure 6.2 Firmness and tensile strength measurement by texture analyser.	120
Figure 7.1 Amount of reducing sugar released during <i>in vitro</i> digestion for control (C), and pasta containing 5% salmon fish powder (SFP), 10% SFP, 15 % SFP and 20% SFP respectively.....	128

Figure 7.2 Starch content hydrolysed within 20 min readily digestible starch (RDS) left and within 120 min slowly digestible starch (SDS) right of pasta enriched with 5%, 10 %, 15% and 20% SFP. The values are expressed as mean \pm SD (n=3). Different letters showed the significant difference ($P < 0.05$). ...	129
Figure 7.3 Values for area under the curve (AUC) comparing control and enriched salmon fish powder (SFP) pasta samples.....	131
Figure 7.4 The pH vs time curves obtained by pasta made with different concentration of salmon fish powder (SFP) incubated with multi-enzymes (Trypsin, Chymotrypsin and protease).	133
Figure 7.5 Total phenolic content of pasta enriched with different concentration of (SFP), before digestion and at gastric and pancreatic digestion. Bar represent mean \pm SD (n=3), followed by different small (before digestion), capital (gastric) and small underlined (pancreatic digestion) letters indicate significant difference among the values at $P < 0.05$	136
Figure 7.6 Antioxidant activity of pasta enriched with SFP determined with ORAC assay during <i>in vitro</i> gastric and pancreatic phase of digestion and before digestion. Results are expressed as Trolox ($\mu\text{mol/g}$). Data are mean \pm SD (n=3), followed by small (before digestion), capital (gastric) and small underlined (pancreatic digestion) letters indicate significant difference among the values at $P < 0.05$	137
Figure 7.7 Sensory analysis, appearance (A), Colour (B), Aroma (C), Taste (D), Texture (E) and Overall quality (F) of pasta fortified with 5% and 15% cod powder (CP) and salmon powder (SP) and control. The reported values refer to the mean \pm standard deviation. The same letter above the bar indicates no significant difference between samples ($P < 0.05$) as determined with Tuke'y test.	141
Figure 9.1 Bread enriched with cod powder.....	166
Figure 9.2 <i>In vitro</i> protein digestibility of bread enriched different levels of cod fish powder (CFP). CB, bread produced with semolina; CB5, CB10 and CB15, bread with 5,10, and 15 g CFP/100 g of cod powder. Results are the means of 3 measurements \pm standard deviations (n=3).	170
Figure 9.3 <i>In vitro</i> starch digestibility of bread. (a) Amount of reducing sugars released (mg/g food) during <i>in vitro</i> digestion. (b) Values for area under the curve (AUC) comparing the control bread. Control bread (CB); CB5, CB10 and CB15: bread produced with 5, 10 and 15 g cod fish powder/100 g	

semolina flour. Results are the mean of 3 measurements \pm standard deviations (n=3). ^{a to d} Values with different superscript letters differ significantly ($P < 0.05$).	172
Figure 9.4 Total phenolic content (mg Gallic acid/g) and radical scavenging activity on DPPH radical of breads. CO, bread made with the semolina; CB5, CB10 and CB15, bread produced with 5%, 10% and 15% cod fish powder. Results are the mean of three measurements \pm standard deviations (n=3). ^{a to c} Values with different superscript letters differ significantly ($P < 0.05$).	174
Figure 10.1 Texture analyser with probe for measurement of texture quality of bread.	183
Figure 10.2 Bread enriched with different levels of salmon powder.	185
Figure 10.3 <i>In vitro</i> protein digestibility of bread enriched different levels of salmon fish powder (SFP). Control bread; 5% SFP, 10% SFP and 15% SFP: bread produced with 5, 10 and 15 g salmon fish powder, 100 g wheat flour. (n=3 \pm standard deviation).	189
Figure 10.4 <i>In vitro</i> starch digestibility of bread. (a) Amount of reducing sugars released (mg/g starch) during <i>in vitro</i> digestion. (b) Values for are under the curve (AUC) comparing the control bread. Control bread; 5% SFP, 10% SFP and 15% SFP: bread produced with 5, 10 and 15 g salmon fish powder, 100 g wheat flour. (n=3 \pm standard deviation). ^{a to d} Values with different superscript letters differ significantly ($P < 0.05$).	193
Figure 10.5 Total phenolic content (mg Gallic acid/g) and radical scavenging activity on DPPH radical of breads. Control bread; 5% SFP, 10% SFP and 15% SFP: bread produced with 5, 10 and 15 g salmon fish powder, 100 g wheat flour. (n=3 \pm standard deviation). ^{a to c} Values with different superscript letters differ significantly ($P < 0.05$).	195
Figure 11.1 LCM-Raman spectroscopy of WS, WS-WG and TCM samples. Sample code: Wheat starch (WS), SO: salmon oil; CONT: Coconut oil; wheat gluten (WG).....	207
Figure 11.2 FTIR spectra of WS, WS-WG and TCMs samples. Sample code: WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten	208
Figure 11.3 XRD spectra of WS, WS-WG and TCMs samples. Sample code: WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten.	210

Figure 11.4 Amount of reducing sugar released during <i>in vitro</i> digestion for wheat starch and replacement of wheat starch with 15 % cod oil, salmon oil, coconut oil and 10 % wheat gluten.	211
Figure 11.5 NMR spectra of WS, WS-WG and TCMs samples. Sample code: WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten	213
Figure 11.6 Scanning electronic microscopic images of wheat starch (WS) and its blends with 15% salmon oil (SO), cod oil (CO), coconut oil (CONT) and 10% wheat gluten (WG).....	215
Figure 12.1 LCM-Raman spectroscopy of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).....	227
Figure 12.2 FTIR spectra of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).	228
Figure 12.3 XRD spectra of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).	229
Figure 12.4 Amount of reducing sugar released during <i>in vitro</i> digestion for wheat starch and replacement of wheat starch with 15 % cod oil, salmon oil and coconut oil.	231
Figure 12.5 NMR spectra of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).	232
Figure 12.6 Scanning electronic microscopic (SEM) images of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).	234

Chapter 1

Introduction

1.1 Background

Recently the World Health Organisation (WHO) reported that 68% of deaths appear related to cardiovascular disease, cancer, inflammation, and respiratory disease which are caused due to the growth of societies, rapid industrialisation, modern lifestyles and urbanisation. In addition, WHO mentioned that globally 422 million peoples are affected with diabetes (World Health Organization (WHO), 2016). Now-a-days the popularity of convenience food products has increased due to its ease of preparation, shelf life stability, nutritive value and textural quality (Brennan, Derbyshire, Tiwari, & Brennan, 2013b). Cereal based foods prepared from corn, rice and wheat are widely consumed and represent a key part of the global diet. They are rich in carbohydrate and low in protein. However, the composition and processing of these cereal foods mean that they possess high levels of rapidly digestible starch which in turn raises nutritional and health issues (Almanza-Benitez, Osorio-Diaz, Mendez-Montecalvo, Islas-Hernandez, & Bello-Perez, 2015) and leads to suggestions that high starch food should be fortified with proteins and lipid to reduce the glycaemic impact of such foods and provide a balanced nutritional profile. Various studies have been conducted, using different protein sources, in the fortification of cereal foods including soy (Mesa et al., 2009), legumes (Pastor-Cavada et al., 2011), and chickpea and lentil (Zhao, Manthey, Chang, Hou, & Yuan, 2005). Others, aimed to increase ω -3 polyunsaturated fatty acid (PUFA) content of the food by including seaweed (Prabhasankar et al., 2009). The nutritional value of such fortification with proteins and lipids is dependent not only on the quantity

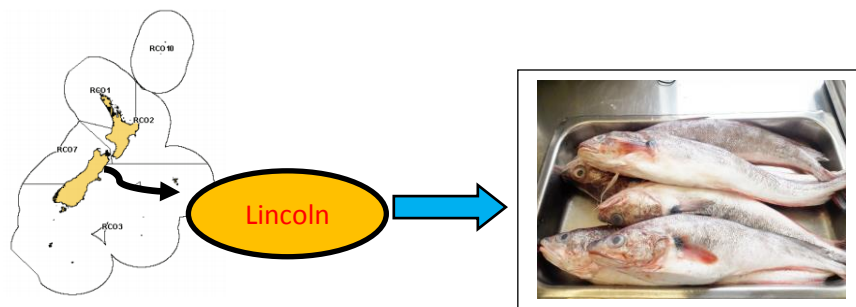
of protein and lipid incorporated but also the quality of such proteins and lipids used, their potential digestibility and ability to form a complex with other food ingredients and the subsequent bioavailability. Quality may refer to the availability of a range of different amino acids and fatty acid profile and the overall ease of digestibility of protein. Proteins and lipids are major structural and metabolic constituents of plant and animal materials and are important source of dietary amino acids, fatty acids as well as functional components of foods (Wolfe, Baum, Starck, & Moughan, 2017). Animal proteins are different in composition to those of plant proteins. For instance, the proteins of the major cereals and legumes are often deficient in at least one of the essential amino acids. While proteins of cereals, such as rice, wheat, barley, and maize are very low in lysine and rich in methionine, those of legumes and oilseeds are deficient in methionine and rich (or adequate) in lysine. Some oilseed proteins, such as peanut protein are deficient in both methionine and lysine contents. The essential amino acids whose concentrations in a protein are below the levels of a reference protein are termed limiting amino acids (Gobbetti & Ganzle, 2013). The nutritional quality of foods made from proteins and lipid which are deficient in an essential amino acid and fatty acids may be improved by incorporating other protein and lipid sources which are rich in essential amino acid (Nogueira & Steel, 2018) and fatty acids (Rodriguez De Marco, Steffolani, Martinez, & Leon, 2018). Fish is a rich source of high quality protein, ω -3 fatty acids, vitamins and minerals. It contains the essential amino acids, particularly methionine, lysine, histidine, threonine and valine which is in contrast to most protein from the plant sources such as wheat, rice, maize, barely, soybean and pea, which lack adequate amounts of one or more essential amino acids Tacon & Metian (2013). The WHO recommended that fish protein should be regarded as a significant source of

essential amino acids (about 30% by weight) (Hankey, 2003). Research has illustrated that the nutritive value of fish protein is better than that of casein and animal protein such as milk and beef because of its favourable essential amino acid content (Gilbert, Bendsen, Tremblay, & Astrup, 2011). Most of the cereal foods are restricted in lysine content. Limited supply of lysine in the diet can lead to mental and physical disability as the lysine is main precursor of the synthesis of glutamate which is the most significant neurotransmitter in the nervous system of mammals (Jarome & Lubin, 2013). Thus, there is increased attention on how fortification of cereal based foods with protein rich ingredients can benefit the nutritional quality. In recent years, customers have become more aware of the diet and health resulting in an increased healthier food with a rise in interest in functional and nutritional foods (Brennan, Merts, Monro, Woolnough, & Brennan, 2008). Research focusing on the development of cereal based products from the fish protein ingredients including dried fish protein is increasing globally (Shaviklo, Thorkelsson, Sveinsdottir, & Pourreza, 2013). The composition of the fish proteins, and associated ingredients, has been shown to have beneficial effects on human health including decreased cardiovascular risk and anti-carcinogenic properties, lower insulin resistance and improved hyperglycaemia, decreases brain glucose and hyper peroxidation (Madani, Malaisse, & Ait-Yahia, 2015). Fish is also significant source of long chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA, C20:5 ω -3), docosahexaenoic acid (DHA, C22:6 ω -3), and arachidonic acid (C20:4 n-6) which are not synthesised by human body but their inclusion in human diet is essential (Kolanowski & Laufenberg, 2006). In general, lipids providing flavour, aroma colour, texture and nutritive value to the product. It also provides energy and help to carry fat soluble vitamins A D, E and K which maintain the integrity of the tissue membrane and

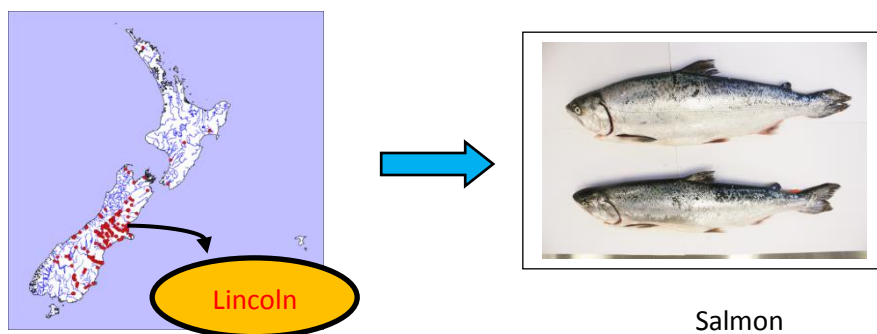
regulate inflammation and blood clotting (Kris-Etherton, Harris, & Appel, 2002). Recently, research has concentrated on utilisation of protein and lipid rich ingredients such as fish protein to enhance the nutritional quality of the product and lowering the blood glucose level and increase the PUFA (Weichselbaum, Coe, Buttriss, & Stanner, 2013). Consumer awareness and the food industry has focused on use of fish protein in products, including ice cream, biscuits, mayonnaise and crackers due to its high quality protein and lipid content, hence it may be useful in the development of functional foods (Shaviklo, 2013). Cereal products such as pasta and bread enriched with legumes and animal proteins have been frequently studied but little research has been carried out on the supplementation of fish protein powder in pasta and bread and its effect on the textural properties, nutritional and sensory characteristics.

This study focused on the use of two kind of fish protein powders prepared from Red cod fish (*P. bachus*) and salmon fish (*O. tshawytscha*) and its incorporation into two types of cereal food products, including fresh semolina pasta and bread by utilising cold extrusion and oven bake methods. Red cod belongs to the Moridae family and is generally found throughout the New Zealand maritime zones, however they are particularly abundant along the east coast and in the Canterbury region. Usually found in shoals, they migrate from the outer continental shelf to shallow coastal grounds. The colour of fish is red-brown above, becoming pink on the sides and belly. The dorsal and anal fins are pink with black margins, and there is a black spot at the pectoral fin base. The fish generally grow up to 40-70 cm long and have an average weight of 1-2 kg. Salmon, also called King salmon, belongs to the Salmonidae family. The average length of salmon ranges from 40-100 cm and the average weight is between 8-10 kg, however it may reach up to 30 kg. They are a pelagic fish species which feed on a wide range of

terrestrial and aquatic invertebrates as well as small fish. Salmon occur mainly on the east coast of the South Island from the Waiau River in the north to the Clutha River in the south. The colour of salmon is a bright silver with a greenish olive back with small black spots.



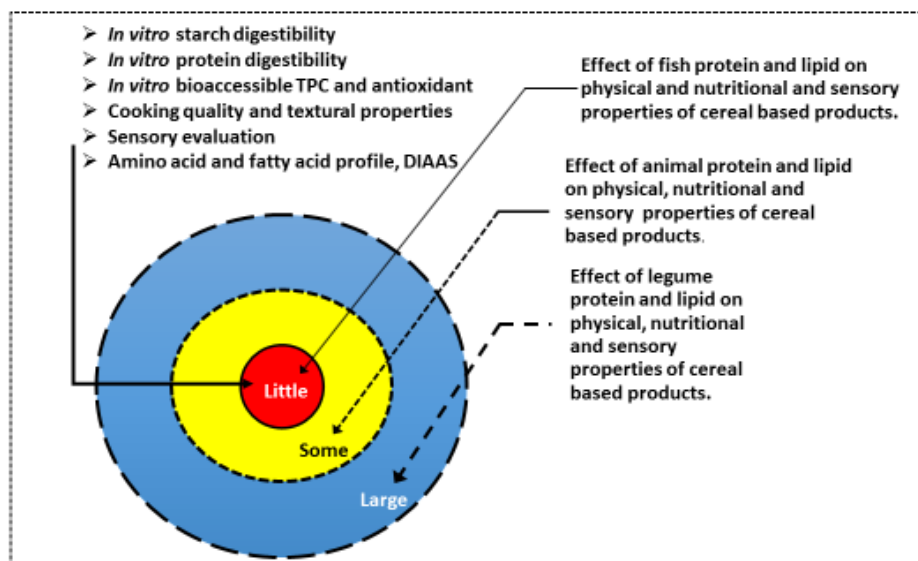
Red cod



Salmon

The objective of this work was to determine the influence of inclusion of fish protein powder on the physiochemical, nutritional and sensory quality of pasta and bread. This research demonstrated the possibility to develop cereal products with increased protein, amino acid, fatty acid contents and protein digestibility and a reduced glycaemic response. The effect of addition of fish protein powder on antioxidant capacity and sensory quality of the products was evaluated. This nutritionally enriched product will be helpful to avoid the development of many chronic diseases and will be a value added product.

1.2 Research gap



A large number of researchers have conducted studies on the incorporation of legume protein and lipid into cereal based products (pasta and bread), including faba bean flour (Petitot, Boyer, Minier, & Micard, 2010), sorghum flour (Khan, Yousif, Johnson, & Gamlath, 2013), aleurone flour (Bagdi et al., 2016), flaxseed flour (Marpalle, Sonawane, & Arya, 2014) and plant oil (Lau, Zhou, & Henry, 2016) to enrich the nutritional properties and discover the physical effects. Cereal products have also been fortified with dietary fibre and animal protein to enhance the nutritional properties (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015a; Liu et al., 2016). Currently, little work has been reported on enriching pasta and bread products with fish protein powder obtained from cod and salmon fish and its effect on cooking quality characteristics, glycaemic response, protein digestibility, *in vitro* bio-accessible antioxidant properties and sensory evaluation.

1.3 Aim of research

The aim of this research was to use fish protein powder as a value added ingredient from cod fish (*P. bachus*) and salmon fish (*O.tschawytscha*) to enrich the cereal based products (fresh pasta and bread). The use of protein rich ingredients in cereal products may help to reduce disease and provide nutritionally rich and healthy product. The effect of incorporation of fish protein powder on physicochemical, nutritional and sensory characteristics of pasta and bread products were analysed. In order to achieve the main aim, the following objectives were designed

1.4 Objectives

1. To develop fish protein powder from cod fish (*P.bachus*) and salmon fish (*O. tschawytscha*) and assess their proximate composition, amino acid profile and fatty acid composition.
2. To develop cereal products (pasta and bread) from fish powder and determine their physico-chemical and textural characteristics.
3. To determine the protein quality of enriched pasta and bread in terms of amino acid profile, fatty acid composition and digestible indispensable amino acid score (DIAAS).
4. To analyse the glycaemic response, protein digestibility and antioxidant properties of these fish powder enriched cereal products.
5. To evaluate the effect of inclusion of fish protein powder into cereal product on sensory quality.

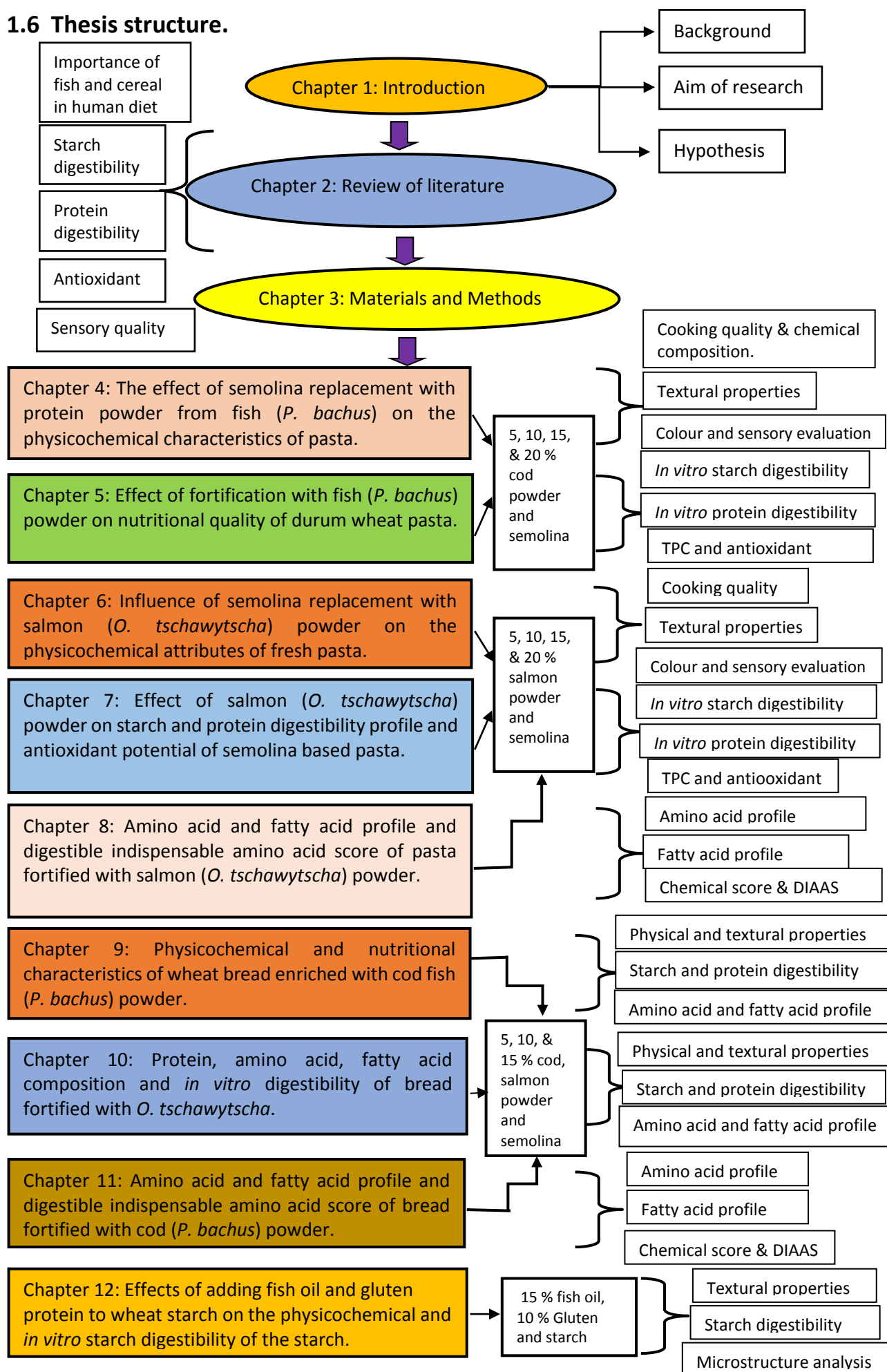
1.5 Hypothesis

Cereal products are often deficient in at least one of the essential amino acids, ω -3 fatty acids and low in protein content. Fish is a rich source of quality protein consisting of

well balanced essential amino acids, micronutrients and ω -3 essential fatty acids that increase the digestibility of protein and effect on human health. There is limited literature to be found that describes effect of fish protein powder on physico-chemical and nutritional properties of cereal products. Therefore, this research hypothesize that,

1. Inclusion of fish protein powder from cod (*P. bachus*) and salmon fish (*O. tshawytscha*) will have a positive effect on the physical and textural properties.
2. Addition of fish protein powder will increase protein content, amino acid and fatty acid profile of the enriched cereal products.
3. Addition of fish protein powder will decrease the starch digestibility and increase the protein digestibility of the enriched products.
4. Incorporation of fish protein powder will increase the phenolic and antioxidant of the products.
5. Addition of fish protein powder will have positive effect on the sensory evaluation up to desire level of addition.

1.6 Thesis structure.





Chapter 13: General discussion and conclusion



Chapter 14: References

Chapter 2

Review of Literature

This chapter summaries information from current literature and provide a critical evaluation in relation to the use of fish protein powder to enhance the physicochemical, technological, nutritional and sensory quality of cereal food products like pasta and bread. The chapter includes observations about previous research findings and results from using fish protein powder to influence the cooking quality, glycaemic impact, protein content and digestibility, total phenolic content, antioxidant capacity and sensory quality of pasta and bread products. The details of the importance of fish consumption in human nutrition and the effect of cold extrusion and baking processing mechanism on protein and starch digestibility on pasta and bread products are discussed. In recent years, consumers have become more aware of the diet and health resulting in an increase of healthier food with a rise in interest in functional and nutritional foods (Brennan et al., 2013b). Research focusing on the development of convenience food from protein rich ingredients including dried fish protein is growing. Fish is leaner than red meat, lower in fat content and rich source of amino acids and essential fatty acids and also contains significant levels of minerals which are vital to the body (Kadam & Prabhasankar, 2010). The composition of the fish proteins and associated ingredients has been shown to have beneficial effects on human health including decreased cardiovascular disease risk and anti-carcinogenic effects in cancer patients (Tacon & Metian, 2013). Cereals are rich in carbohydrate and low in protein. However, the composition and processing of cereal based products mean that they possess high levels of rapidly digestible starch which in turn raises nutritional and health

issues (Cho & Rizvi, 2010). Therefore this thesis evaluates the replacement of semolina and wheat flour with red cod (*P. bachus*) and salmon (*O. tshawytscha*) fish powder in pasta and bread.

2.1 Importance of fish consumption in human diet

The world per capita fish consumption has increased from an average of 9.9 kg in the 1960s to 20 kg in 2015 (FAO, 2016). The level of consumption varies depending on the country and continent, in 2010, the per capita fish consumption values in Oceania, North America, Europe, and Latin America and Caribbean were 25.4 kg, 21.8 kg, 22.0 kg, and 9.7 kg respectively. Urbanization, and the growing concern over healthy eating habits in developed countries as well as the increase in the purchasing power of developing countries, have contributed to an increase in the worldwide fish demand (Graziano, 2014). More than 795 million people had chronically inadequate levels of dietary intake during 2014-15 (McGuire, 2015). According to the report, 1.6 billion people were affected by iron deficiency and anaemia, while about 1.5 billion people were overweight with half billion obese and that results in a greater risk of cardiovascular disease and other non-communicable diseases (Tacon & Metian, 2018). Fish represent an important component of the human diet, providing about 3.1 billion people with almost 20 % of their average daily animal protein intake (FAO, 2016), and providing readily available dietary source of LC-PUFA (ω -3) for direct human consumption Including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Tacon & Metian, 2018). Fish have a high nutritional value regarding beneficial amounts of protein, lipids as well as essential micronutrients. They are a rich source of high quality protein and ω -3 long chain polyunsaturated fatty acids (Tacon & Metian, 2013).

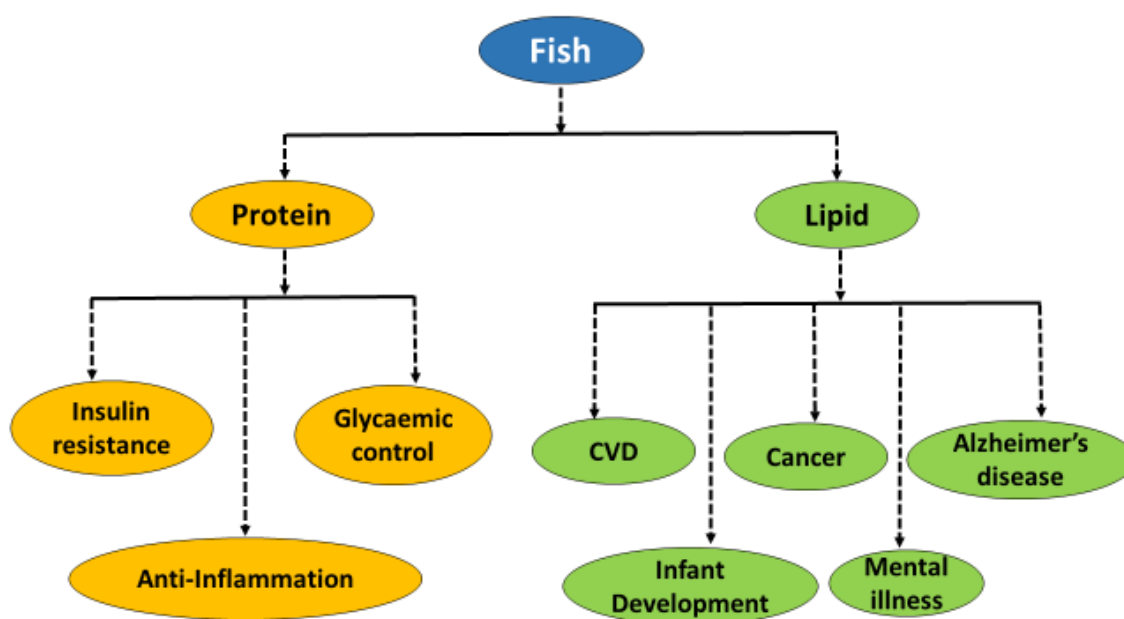


Figure 2.1 Importance of fish protein and lipid in human health

Several scientific studies have confirmed that the positive effect of consumption of lean (cod) and fatty (salmon) fish and the reduction in outbreaks of many chronic disease related to human health including, a reduction in the sudden cardiac death and a decrease in the occurrence of cardiovascular disease (Baum et al., 2012; Lund, 2013), reduction in chronic inflammation (Lund, 2013), a reduction in postprandial glycaemic response (Aadland et al., 2016) and insulin resistance (Kalupahana et al., 2010), aiding infant growth (Connor, 2000), decrease the occurrence of cancer (Wu et al., 2012) and Alzheimer's disease (Raji et al., 2014) as shown in Figure 2.1. Kalupahana et al. (2010) showed that *in vivo* treatment of eicosapentaenoic acid (EPA) can prevent and improve insulin resistance by mice fed with a high fat diet. Further, proteomic studies have indicated that dietary EPA supplementation of high fat diets was associated with reduced adipose inflammation and lipogenesis and elevated markers of fatty acid oxidation. The glycaemic index (GI) is a method that is used to predict the postprandial blood glucose level in the food matrix (Foschia, Peressini, Sensidoni, Brennan, &

Brennan, 2015b). Foods with a high GI are rapidly digested in the human body resulting in a marked increase in the blood glucose levels and a greater insulin demand. Over consumption of high GI foods may increase the risk of metabolic disorders such as type 2 diabetes, cardiovascular disease and obesity (Kim et al., 2008). Research carried out by Wallin et al. (2017) showed that lean and fatty fish consumption may be beneficial for reducing the risk of diabetes, and thus recommended regular fish consumption. Aadland et al. (2016) reported that consumption of lean fish reduces postprandial concentrations of C-peptide, lactate and the total glycerol (TL)/ high density lipoprotein (HDL)-cholesterol ratio in healthy adults which is useful for preventing the development of long-term insulin resistance. Siriwardhana, Kalupahana, & Moustaid-Moussa (2012) reported that ω -3 PUFA was able to inhibit the oxygenation of arachidonic acid by cyclooxygenase and generated much lower prostaglandin effect thus imparting significant beneficial effects against inflammation. It has also been shown that EPA and DHA can suppress the cyclooxygenase activity in human (Soon et al., 2009). Consumption of 300-400 g of cod and salmon per week was found to have beneficial effects on weight control, blood pressure and reduced outbreaks of chronic inflammation (Van Den Elsen et al., 2011). Presently, the leading cause of death in the world is cardiovascular disease (CVD). There is a valid evidence of an inverse relationship between the amount of ω -3 fatty acids in the food and in blood cell and CVD diseases. Although, saturated fats and cholesterol are harmful for the heart, ω -3 fatty acids EPA and DHA and from fish are beneficial and are protective against CVD disease. These beneficial long chain polyunsaturated fatty acids (LC-PUFA) are abundant in fish and absent in cereals (Connor, 2000).

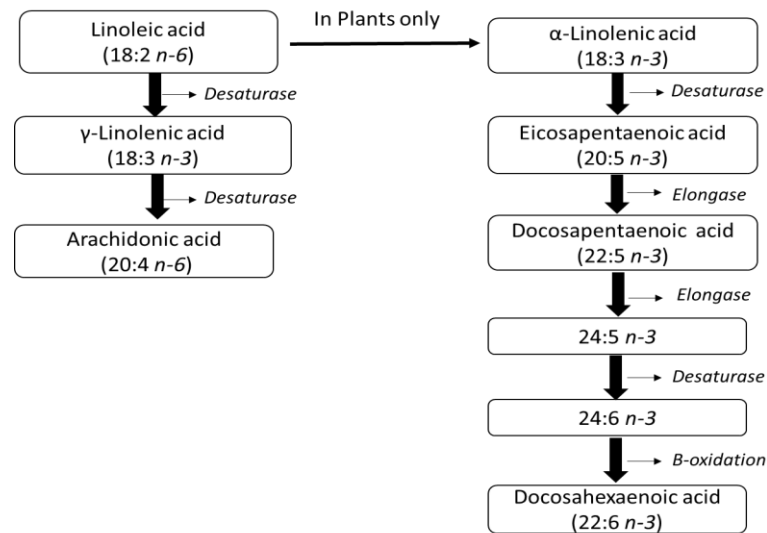


Figure 2.2 Conversion of linoleic and α -linolenic acids into their longer chain derivatives.

The LC-PUFA (ω -3 and ω -6) play an important role in many biological activities and are derived from linoleic acid (LA) and α -linolenic acid (ALA). LA and ALA cannot be produced in the human body but have to be supplied by dietary sources. LA is mainly converted to arachidonic acid (AA) but can also be converted to docosapentaenoic acid (DPA), while ALA is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are major components of the human cell membrane (Figure 2.2). However this conversion in humans is inefficient and these fatty acids could be easily increased by consuming fish or ω -3 supplements of good quality (Sanders, 2010). Arachidonic acid acts as a substrate for the synthesis of prostaglandins, thromboxanes and leukotrienes which regulate smooth muscle contraction, platelet aggregation, inflammation, and immune function. However, it has been recorded that LC-PUFA are consumed in relatively small amounts in the Western diet. Around half of the New Zealand adult population have a fat intake above the recommended (33%) upper level. In particular, SFA intake was 13 %, which means more than half of NZ adults have an intake above the recommended level, whereas more than half of the NZ adult population has a PUFA

intake (4.9%) below the minimum recommended level (6-11%). In order to reduce their risk of CVD, it would be beneficial to replace some SFA in the NZ diet with PUFA (Winsome Parnell & Mackay, 2011). The FAO recommends a daily intake of 250 mg EPA plus DHA and higher intake of 300 mg/day for pregnant and lactating women (FAO/WHO, 2010). Results published by Mori et al. (2000) indicated that an intake of 4 g/day of EPA decreased triglyceride levels by 23%. Also, Kris-Etherton, Harris, & Appel (2002) reported that supplementation with 2-4 g/day of EPA+DHA can lower plasma triglycerides levels by 25-30%. These outcomes are supported by Marik & Varon (2009) who reported that intake of EPA and DHA resulted in a significantly reduced risk of cardiovascular death, risk of sudden cardiac death and a significantly reduced risk of mortality. Several researchers have found a significant inverse association between fish consumption and the risk of stroke, with those with the highest consumption of fish having a 12-13 % lower risk of stroke compared to those with the lowest intakes (Xun et al., 2012). The link between fish intake and pancreatic and prostate cancers has been investigated and it was suggested that LC-PUFA has anti-inflammatory properties which may reduce the risk of pancreatic (Farrow & Evers, 2002) and prostate cancer (Szymanski, Wheeler, & Mucci, 2010).

The changes in dietary protein and fat intake, in particular increased intakes of n-6 PUFA through cereals and decreased intakes of LC-PUFA, have contributed to an increase in asthma allergic disease (Black & Sharpe, 1997). A suggested mechanism is that in the cell membrane the levels of arachidonic acid (n-6 PUFA) is increased with an increase in the ω -6: ω -3 ratio, which in turn leads to an increased synthesis of prostaglandin E₂ that is involved in allergic reactions (Sala-Vila, Miles, & Calder, 2008). Sources of ω -3 and n-6 fatty acids from plant and animal based foods are different and depicted in Table 2.1.

Table 2.1 Quantity of EPA and DHA in selected food (g/100g)

Food	ω -3		ω -6	
	EPA	DHA		EPA:DHA
Cod (Raw)	0.08	0.14	-	0.57
Salmon (Raw)	0.86	1.10	-	0.78
Beef	0.15	0.03	0.38	5
Lamb	0.00	0.36	0.01	0.036
Seeds (Chia and hemp seed)	-	-	5.84 27.36	-
Salmon oil	13.20	18.23	-	0.72
Cod liver oil	9.90	10.97	-	0.90
Wheat flour*	-	-	57.91	-
Semolina**	-	-	58.60	-

Source: USDA National Nutrient Database for Standard Reference (<http://ndb.nal.usda.gov/ndb/search>, accessed on 18th July, 2018).

*(Nikolic, Sakac, & Mastilovic, 2011)

** (Fradique et al., 2013)

Table 2.1 shows the ω -3 (DHA and EPA) and n-6 (linoleic acid) fatty acids content of various foods (fish and cereals). It can be seen that fish provides proportionally more DHA and EPA than beef, lamb and cereals. Weichselbaum, Coe, Buttriss, & Stanner (2013) suggested that a higher fish intake leads to an increase in the level of dietary DHA and DHA in the blood may be positively associated with a lower risk of dementia and Alzheimer's disease. Similar observations were reported by Raji et al. (2014) who worked on supplementation of ω -3 fatty acids and its effect on Alzheimer's disease. Results showed that consumption of fish increased the grey matter volume in the brain areas which is responsible for brain structure improvement and decreased risk of Alzheimer's disease. Fish is a rich source of protein, fat and vitamins which is required for foetal neurodevelopment including amino acids, vitamin D and long-chain ω -3 fatty acids. During the pregnancy iodine levels in women is decreased and this has been associated with adverse effects on offspring. A study carried out by Hibbeln et al. (2007)

reported that fish consumption lower than 340 g/week during pregnancy was associated with an increased risk of being in the lowest quartile for verbal intelligence quotient (IQ) and was also associated with an increased risk of suboptimal outcomes for prosocial behaviour, fine motor, communication, and social development scores. They also suggested that, consumption of two to three portions of fish per week is associated with beneficial effects on child development.

Table 2.2 Recommendations for fish and long-chain ω -3 PUFA intake from different food agency.

Agency	Year	Recommendations
European Food Safety Authority (EFSA) (European Food Safety Authority (EFSA), 2010)	2010	➤ 250 mg EPA + DHA/day or 1-2 portions of oil-rich fish per week. ➤ 100-200 mg DHA during pregnancy and lactation.
World Health Organization (WHO) (FAO/WHO, 2010)	2008	➤ 250 mg EPA + DHA per day for adult men and non-pregnant women.
Food and Agriculture Organisation of the United Nations (FAO) (FAO/WHO, 2010)		➤ 300 mg EPA + DHA per day for pregnant women.
Australia and New Zealand National Health and Medical Research Council (Baghurst, 2005)	2005	➤ 610 and 430 mg/day of DHA/EPA, for men and women respectively.
Scientific Advisory Committee on Nutrition (SACN) (Jackson, Key, & Williams, 2004)	2004	➤ 2 portions of fish a week (equivalent to 450 mg of long-chain ω -3 PUFA per day).
American Heart Association (AHA) (Kris-Etherton et al., 2002)	2003	➤ 2 portions of oily fish/week.

Mental illness disorder is commonly related to brain structure and its fluidity. The brain is composed of 25 % phospholipids as lipid storage organ. ω -3 fatty acids play an important role in the production of anti-inflammatory and aggregatory eicosanoids

which increase the brain function. Supplementation of DHA and EPA in the cell membrane improves brain membrane fluidity that help to bind ligands (Stillwell & Wassall, 2003). Recommendations for the consumption of fish and long-chain ω -3 PUFA have been published by a variety of organisations for prevention of health disease as shown in (Table 2.2).

2.1.1 Importance of protein

Proteins are the most fundamental structural and functional elements of the food matrix and a vital source of amino acids. It is broken down in tissues and contribute to an amino acid pool from which they are reused to synthesize enzymes, hormones, lean tissue, immune function proteins, muscle mass, bone matrix, and other essential compounds. It is important to consume the recommended dietary protein (0.8 g protein /kg body weight/day for a healthy adult human) for the synthesis and maintenance of body mass and muscle function (Wu, 2016). Table 2.3 illustrates the recommended dietary protein requirements for different age groups of human that is important to regulate human physiology (FAO/WHO/UNU, 2007). Studies carried out by Hankey (2003) and Wu (2016) suggest that the significant levels of protein intake in the diet help in reducing bodyweight, improve resistance to infectious disease, blood circulation, foetal growth and skeletal muscle.

Table 2.3 Recommended dietary protein requirements by humans of all age groups.

		Dietary protein requirements (g protein/kg body weight/day) (FAO/WHO/UNU, 2007)	
Group	Age (years)	1985	2007
Infants	0.5	1.75	1.31
Children	1-2	1.18	1.02
	3-10	1.05	0.92
Adolescents	11-14	0.99	0.90
	15-18	0.97	0.87
Adults	>19	0.86	0.83

At present, worldwide, one billion people have inadequate protein intake contributing to impaired growth and development. The animal based food contain relatively high levels of protein (> 40 % on dry matter basis) while cereal contains < 15 % (dry matter basis) protein. Additionally, most cereal proteins are incomplete as they are deficient in one or more essential amino acids (Wu, 2013). Animal protein based food contains balanced amounts of all essential amino acids that promote optimal growth, development and health. It supplies taurine (sulphur containing amino acids) which is beneficial for protecting the eyes, heart, skeletal muscle and is essential for reduced oxidative damage and degeneration (Wu et al., 2013). Layman et al. (2005) reported that a high protein and low carbohydrate intake diet helped to decrease the body fat and improve metabolic profile in an obese group as compared to the food with low protein. Josse, Atkinson, Tarnopolsky, & Phillips (2011) showed that a higher protein intake food resulted in the loss of body fat and preservation of muscle mass. A possible reason for this is that supplementation of protein in the diet may increase the levels of arginine circulation, which enhances insulin sensitivity, stimulates the oxidation of fatty acids and glucose in skeletal muscle. This then promotes whole body energy utilisation,

and reduces white-fat mass in obese humans (McKnight et al., 2010). Table 2.4 illustrates requirements for essential amino acids by the human body.

Table 2.4 Requirements of essential amino acids (EAA) (mg amino acid/kg body weight/day) by the infants, children, adolescents and adults (FAO/WHO/UNU, 2007).

Amino acids	Infants	Children		Adolescents		Adults
	0.5 (yrs)	1-2(yrs)	3-10 (yrs)	11-14 (yrs)	15-18 (yrs)	>19 (yrs)
Histidine	22	15	12	12	11	10
Isoleucine	36	27	22	22	21	20
Leucine	73	54	44	44	42	39
Lysine	63	44	35	35	33	30
Methionine + cysteine	31	22	17	17	16	15
Phenylalanine + tyrosine	59	40	30	30	28	25
Threonine	35	24	18	18	17	15
Tryptophan	9.5	6	4.8	4.8	4.4	4.0
Valine	48	36	29	29	28	26

Proteins are major structural and metabolic constituents of plant and animal materials and are important source of dietary amino acids as well as functional components of foods (Sosulski & Imafidon, 1990). Animal proteins are different in composition to those of plant proteins. For instance, the proteins of the major cereals and legumes are often deficient in at least one of the essential amino acids. While proteins of cereals, such as rice, wheat, barley, and maize are very low in lysine and rich in methionine, those of legumes and oilseeds are deficient in methionine and rich or adequate in lysine. Some oilseed proteins, such as peanut protein are deficient in both methionine and lysine contents. The essential amino acids whose concentrations in a protein are below the levels of a reference protein are termed limiting amino acids. The essential amino acid contents of various food proteins are described in Table 2.5. Adults consuming only

cereal proteins or legume proteins have difficulty maintaining their health due to the lack of some of these essential amino acid. Children below 12 years of age on diets containing only one of these protein sources cannot maintain a normal rate of growth. The nutritional quality of foods made from proteins which are deficient in an essential amino acid may be improved by incorporating other protein sources which are rich in the essential amino acid (Tieland, Borgonjen-Van Den Berg, Van Loon, & de Groot, 2015).

Table 2.5 Essential amino acid contents and nutritional value of proteins from various sources (mg/g protein) (Friedman, 1996; Sosulski & Imafidon, 1990).

	Protein Source									
Parameter (mg/g Protein)	Egg	Milk	Beef	Wheat	Rice	Maize	Barley	Soy- bean	Pea	Fish
<i>Amino acid concentration (mg/g protein)</i>										
Histidine	22	27	34	21	21	27	20	30	26	35
Isoleucine	54	47	48	34	40	34	35	51	41	48
Leucine	86	95	81	69	77	127	67	82	70	77
Lysine	70	78	89	23	34	25	32	68	71	91
Threonine	47	44	46	28	34	32	29	41	36	46
Tryptophan	17	14	12	10	11	6	11	14	9	11
Valine	66	64	50	38	54	45	46	52	41	61
Methionine	57	33	40	36	49	41	37	33	22	40
+Cysteine										
Phenylalanine	93	102	80	77	94	85	79	95	69	76
+										
Tyrosine										
Total EAA	512	504	480	336	414	422	356	466	394	485
<i>Protein Content (%)</i>										
	12	3.5	18	12	7.5	-	-	40	28	19

Fish proteins are rich in all the essential amino acids (particularly methionine, lysine, histidine, threonine and valine) which is in contrast to most protein from plant sources

such as wheat, rice, maize, barely, soybean and pea, which lack adequate amounts of one or more essential amino acids. The World Health Organization recommended that fish protein should be regarded as a significant source of essential amino acids (about 30% by weight) (Khalili Tilami & Sampels, 2018). Research has illustrated that the nutritive value of fish proteins is better than that of casein and animal protein such as milk and beef because of their favourable pattern of essential amino acid (Weichselbaum et al., 2013). Most cereal foods have limited lysine compared to fish. A limited supply of lysine in the diet can lead to mental and physical disability as lysine is the main precursor of glutamate which is the most significant neurotransmitter in the mammalian nervous system (Papes, Surpili, Langone, Trigo, & Arruda, 2001). Thus, there is increased attention on how fortification of cereal based foods can benefit the nutrition of consumers of different age groups.

2.2 Protein digestibility

Digestibility is an important attribute which is used to evaluate the nutritional quality of proteins in the food matrix. It is related to the amount of essential amino acids (EAAs) present in the food product and the requirements for EAAs by an organism in a specific physiological state, and the bioavailability of the EAAs upon ingestion. Food proteins derived from different sources can be mixed to improve the protein quality and overall amino acid balance in the final food product. In regard to protein sources, partial replacement of fish protein with cereal proteins has gained considerable interest due to improved sustainability and potential health benefits (Joehnke et al., 2018). In the protein digestion process, protein rich food is broken down into amino acids. The catalytic breaking of the peptide bond of food matrix is carried out by enzymes (protease, trypsin and chymotrypsin), which act initially in the acid environment ($\text{pH} <$

4) of the stomach and the process is completed in the alkaline environment of the small intestine (pH 8). The proteolytic enzymes (protease, trypsin and chymotrypsin) selectively attack specific bonds of the protein (Figure 2.3) and break down into amino acids, dipeptides or small oligopeptides. Absorption of these amino acids takes place in the small intestine through specific transporters (Hankey, 2003).

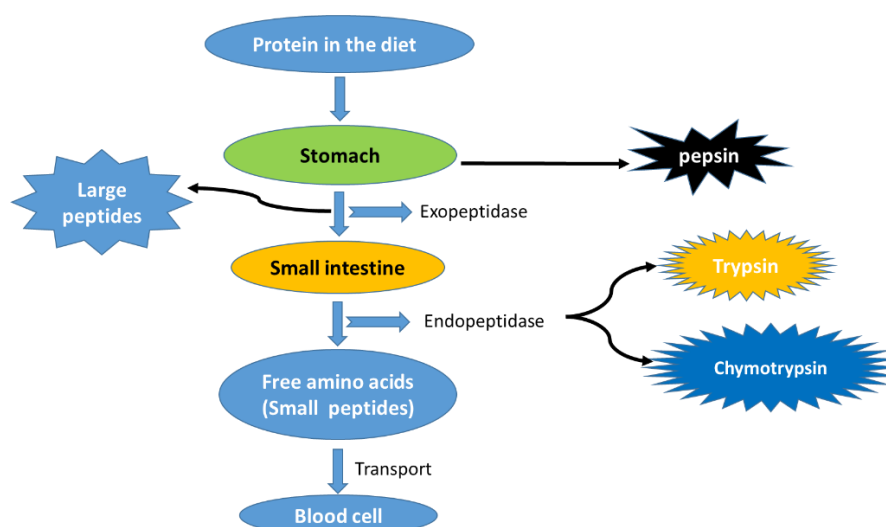


Figure 2.3 Dietary protein intake and its digestion by different enzyme in the gastrointestinal tract.

Trypsin has the ability to cleave the peptide bond to lysine and arginine at the primary binding position with greater specificity. Chymotrypsin enzyme cleaves leucine, tyrosine, phenylalanine, tryptophan, glutamine, serine and threonine amino acids in the small intestine (Aryee & Boye, 2016). Cereal protein has a low digestibility, compared to animal protein, which together with a limited amount of some amino acids (lysine, methionine, cysteine and tryptophan), indicates their low nutritional value (Carbonaro, Maselli, & Nucara, 2012). The protein quality of the fish based products depends mainly on their amino acid composition and digestibility (Deng, Luo, Wang, & Zhao, 2015). Fish is a rich source of easily digestible protein. Fish proteins are highly sensitivity to

proteolytic digestion with a digestibility of more than 90 %. The *in vivo* digestibility of proteins of raw fish meat has been recorded as between 90-98 % (Venugopal, 1992). Due to the increased consumption of carbohydrate dense food material, obesity, dyslipidaemia, and other metabolic disorders are encountered more frequently.

Table 2.6 Effect of different food components on *in vitro* protein digestibility.

Title	Authors	Finding
Effect of dried yam flour (<i>Dioscorea schimperiana</i>) on cooking quality, digestibility profile and antioxidant potential of wheat based	Djeukeu, Gouado, Leng, Vijaykrishnaraj, & Prabhasankar (2017)	Presence of protein and fibre rich yam flour reduced significantly the protein digestibility as compared to control sample.
Starch and protein analysis of wheat bread enriched with phenolics-rich sprouted wheat flour	Swieca, Dziki, & Gawlik-Dziki (2017)	Due to the interaction of the phenolic compound present in the sprouted wheat flour with digestive enzymes and protein, it decrease the protein digestibility of the bread.
The impact of using chickpea flour and dried carp fish powder on pizza quality	El-Beltagi, El-Senousi, Ali, & Omra (2017)	With the supplementation of carp fish powder, the protein digestibility increased due to the highly digestible amino acids.
Effects of meat addition on pasta structure, nutrition and <i>in vitro</i> digestibility	Liu et al. (2016)	Protein digestibility was not significantly increased by meat addition into pasta up to 30%.
Evaluation of cooking, microstructure, texture and sensory quality characteristics of shrimp meat-based pasta	Kadam & Prabhasankar (2012)	Shrimp meat addition to pasta up to 20% did not significantly affect the protein digestibility.
Influence of freeze-dried shrimp meat in pasta processing qualities of Indian <i>T. durum</i> wheat	(Ramya, Prabhasankar, Gowda, Lalitha, Modi, & Bhaskar, 2015)	Pasta made with freeze dried shrimp meat powder showed decreased protein digestibility as compared to only semolina pasta.

Meat consumption can more adequately meet the minimum amino acid requirements of humans than plants alone (Wu et al., 2014). Importantly, fish is a rich source of a methionine and cysteine amino acids which is essential for protecting the eyes, heart, skeletal muscle, and other tissues from oxidative damage and degeneration additionally its antioxidant properties maintains neurological and muscular function (Wu, 2013). The effect of various food components on protein digestibility described in Table 2.6.

2.3 Review of *in vitro* methods to determine protein digestibility

Over the years, various methods have been developed for assessing the quality of protein by protein digestibility method (Table 2.7). Tavano, Neves, & da Silva Junior (2016) and Aryee & Boye (2016) have reported that many techniques are available to estimate protein digestibility, but every method differs from the others. Generally, the variation in methods depends on the digestion time, the concentration and type of the enzymes and samples used. In 1989, the FAO recommended the use of protein digestibility corrected amino acid score (PDCAAS) method for estimation of protein digestibility. It is based on the true faecal nitrogen digestibility and indispensable amino acids content in the test protein (Schaafsma, 2005). Most recently, the FAO (2013) recommended the digestible indispensable amino acid score (DIAAS) method to determine the protein quality of the food matrix, this is based on the measurement of ileal digestibility of individual amino acids instead of true faecal nitrogen digestibility which does not consider the essential amino acids lost into the colon. On the other hand, *in vitro* methods are reliable and deliver quick results compared to *in vivo* experimentation. The *in vivo* experiments have high costs and a long experimental process (approx. 9 days minimum) as well as their being ethical objections to animal use (Schaafsma, 2012). However, there is need to conduct more research on the protein

digestibility of food to improve the correlation between *in vitro* and *in vivo* methods, as accurately mimicking the physiological digestion process is important. Hsu and Vavak (1977) developed a multi-enzyme technique to evaluate *in vitro* protein digestibility of various samples, such as meat, eggs, dairy and plant based products, with the help of various pancreatic enzymes, including trypsin (1.6 mg), chymotrypsin (3.1 mg) and peptidase (1.3 mg). Protein digestibility was estimated by the pH drop over 10 min. It was shown that an enzyme cleaved the peptide bond of the protein to release the H⁺ ions into the surrounding medium and free carboxyl groups were generated due to a drop in the pH of the product. This method is quick and simple to apply to any food product. A multi-enzyme solution was used to avoid potentially inaccurate results being obtained due to trypsin inhibitors being present in some products and to reduce limitations, such as the prolonged digestion time of a single enzyme system. The results were correlated with *in vivo* results obtained when the same samples were fed to rats over a month. The nitrogen contents of the food products before consumption and the faeces were analysed by a macro Kjeldahl method. It was found that *in vivo* apparent digestibility had a high correlation with the pH drop at 10 min after enzyme addition. This method had a correlation coefficient of 0.90 and a standard error of 1.72 with *in vivo* method. This method also revealed that there is no adverse effect of fat content of the product on the protein digestibility and recommended that it is not necessary to remove the fat content prior to *in vitro* protein digestion analysis. Furthermore, the authors reported that protein digestibility of the food matrix could be improved or decreased by the processing method as this affects the destruction of the protease inhibitor and subsequent opening of the protein structure through denaturation or non-enzymatic browning reaction and thermal processing crosslinking. It was also noted that the

protein digestibility of pasta and bread products with this method were found to be decreased (2-8 %) due to the long term drying, low temperature and heat treatment for pasta processing and due to the Maillard reaction between protein of the food matrix and sugar released from the hydrolysis of starch. More recently, Ma, Boye, & Hu (2017) reported that this method could be used successfully for processed yellow field peas samples to determine the *in vitro* protein digestibility of the products.

Table 2.7 Methods of *in vitro* protein digestibility, PDCAAS and DIAAS.

Method for Measure IVPD	Sample Used	Enzymes Used	Reaction Time	Reference
Protein content in supernatant after enzymatic digestion and before protein content in the sample.	Various food proteins Egg, casein, beef, soybean, wheat flour.	1.5 mg Pepsin and 4 mg pancreatin	27 h	Akeson & Stahmann, (1964)
PH drop over 10 min and measurement by regression equation. (Y=210.46-18X)	Various food products Plant, milk and animal protein.	Trypsin: 1.6 mg, Chymotrypsin: 3.1 mg and Peptidase: 1.3 mg	30 min	Hsu & Vavak (1977)
Protein content in the pellet after enzymatic digestion and total protein content in the sample.	Wheat flour and its products	0.05 mg pepsin and 0.25 mg pancreatin	3 h	Pasini et al., 2001)
Protein loss before and after digestion	Soya protein isolate	Pepsin (4 U/mg on a protein basis) and pancreatin (4 U/mg on a protein basis)	4 h	Chen, Zhao, Sun, & Zhao (2013)
Protein content after digestion before digestion total protein content.	Food products cereals and	300 U/mL pepsin and 0.05g pancreatin	3 h	Swieca, Gawlik-Dziki, Dziki,

	other food protein.			Baraniak, & Czyz (2013)
Essential amino acid (AA) scoring pattern for 1-2 yr. old children and <i>in vitro</i> protein digestibility value. PDAAS= (limiting AA content in the sample protein/AA in reference protein) x protein digestibility	Food ingredients, whole foods, sole source foods and mixed diets	1.5 mg Pepsin and 4 mg pancreatin	24 h	Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson (2015b)
Estimation of digestible dietary indispensable AA in food sample and AA in reference protein. DIAAS= 100 x (mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the indispensable AA in 1 g of reference protein)	Food ingredients, whole foods, sole source foods and mixed diets	Amino acid analysis	36 h	FAO (2013)

2.4 Importance of cereal food

Cereals and their products (such as pasta and bread) play an important role in the human diet. They are rich source of carbohydrates (50-80 %) and provide nutrients as well as energy. Cereals are low (8-12 %) in protein content and deficient in one or more essential amino acids particularly lysine, methionine and threonine. Morens et al. (2003) reported that wheat protein has a lower nutritional quality than milk, soy, pea and lupin proteins. To compensate for this deficiency, pasta and bread products can be enriched with other protein and lipid sources (Dewettinck et al., 2008). Pasta is cereal based food and has become popular worldwide due to its ease of preparation, handling, cooking and long shelf-life. Among wheat, durum wheat semolina is regarded as the best raw-material from which to manufacture pasta. The composition of durum wheat

semolina can be divided into 3 main constituents, the main fraction being starch, varying between 70 and 80 % of the total weight, followed by proteins, reaching up to 15 % of the total weight and the remaining part is composed of small amounts of fibre, lipids, vitamins and minerals (Petitot, Boyer, Minier, & Micard, 2010). The most simple and common method for the production of pasta is through cold extrusion. In this process, the semolina flour is mixed with water, (usually about 30-35% moisture (Brennan, Kuri, & Tudorica, 2004). Mixing is an important part of the pasta making process to ensure homogeneity and diffusion of water into centre of semolina particles to form a dough. After mixing, the dough is extruded under high pressure through a die to obtain desirable pasta shape. During extrusion process, the proteins molecules interact strongly and form a strong gluten network which determines the final cooking and texture quality of the pasta (Bustos, Perez, & Leon, 2015). The major protein fractions in the semolina are glutenins and gliadins. Gluten is responsible for the development of dough during mixing and extrusion, entrapping the starch granules in its network. During processing of pasta these proteins interact with each other and form intra and intermolecular disulphide (SS) bonds that will help to develop the strong viscoelastic gluten network (Lamacchia et al., 2010). This viscoelastic network restricts starch swelling, maintaining the structure of pasta during cooking, and thus preventing cooking losses. During cooking, protein coagulation and starch gelatinisation occur. Due to the faster rate of starch swelling compared to the slower rate of protein interaction a weaker protein network is formed inside the pasta (Petitot, Abecassis, & Micard, 2009).

Bread is a staple food, mainly consist of wheat flour, water, yeast and salt. The bread making process involves three stages: mixing and dough development, fermentation

and baking (Figure 2.4). During mixing, the protein combines with the water and begins to form a gluten network. This network is a continuous, homogenous three-dimensional structure that binds the flour particles together in a 'dough'. During subsequent kneading air bubbles are included in the dough and act as an initial nuclei of the gas bubble.

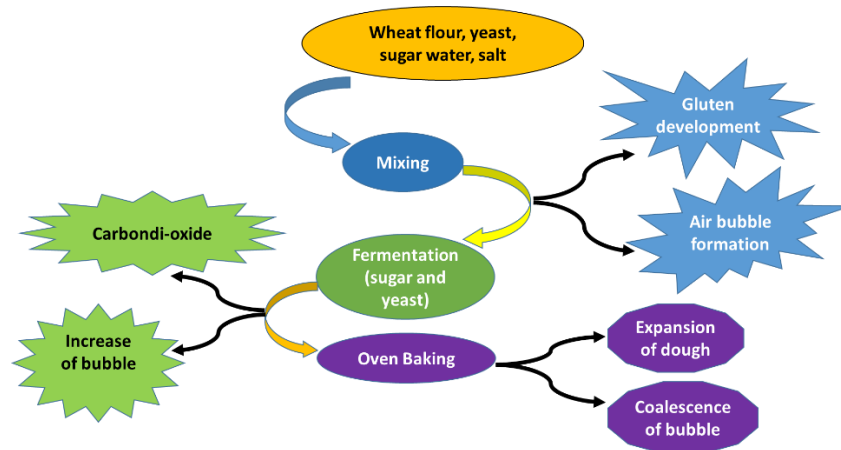


Figure 2.4 Bread making process

The yeast, ferment any available sugars and some of the starch to produce carbon dioxide. This gas is responsible for expanding the dough (Gao, Wang, Dong, & Zhou, 2018). Pasta and bread products can be enriched with exogenous protein and lipid sources from fish to achieve a nutritionally rich final product. Many researchers have reported the effect of protein and lipid addition to pasta and bread and their effects on physiochemical (cooking, texture, colour, volume) and nutritional properties (starch and protein digestibility) (Table 2.8).

Table 2.8 The effect of protein and lipid addition to cereal based products.

Author	Protein and lipid source	Product and substitution level	Effects
Tazart, Lamacchia, Zaidi, & Haros (2016)	Broad bean (<i>Vicia faba</i>) flour	Pasta (10, 30, and 50 %)	Significantly increased protein, fibre, ash and mineral content. <i>In vitro</i> protein digestibility increased and starch digestibility reduced upon broad bean supplementation.
Filipovic, Ivkov, Kosutic, & Filipovic, (2016)	Flaxseed flour	Pasta (10 and 20%)	Improved the ω -6/ ω -3 fatty acid ratio and decreased the texture quality of pasta.
(Laleg, Barron, Sante-Lhoutellier, Walrand, & Micard, 2016)	Gluten(G), Egg powder (EP) and faba bean (FB)	Pasta G: 6 % EP:5 % FB: 35 %	Protein content of pasta increased (13-17%). Protein digestibility of pasta enriched with G and EP reduced (3%) as compared to faba bean made pasta due to highly covalent (28-32%) bonding in pasta structure.
Liu et al., (2016)	Meat emulsion	Pasta (15, 30 and 45%)	Enhanced the gluten network of pasta and increased the firmness and extensibility. It reduced the starch digestibility and increased protein digestibility and amino acid profile of pasta as supplemented with meat emulsion.
Rodriguez De Marco, Steffolani, Martínez, & Leon (2014)	Spirulina	Pasta 5, 10 and 20%)	Increased the protein content of pasta. Glycaemic index and protein digestibility was not affected by spirulina while phenolic content and antioxidant activity increased.
(Ramya et al., 2015)	Shrimp meat	Pasta (2.5, 5 and 10%)	Enhance the firmness and dough stability of pasta. Protein and starch digestibility was reduced while increased the fatty acid and amino acid composition.
Gopalakrishnan, Menon, Padmaja, Sajeev, & Moorthy (2011)	Whey protein (WP) and Sweet potato flour (SPF)	Pasta 50-60% (WP) and 10-20% (SPF)	Reduction in the <i>in vitro</i> starch digestibility with lower rapidly digested starch and highest resistant starch. Protein quality of pasta enhanced with high essential amino acid index (lysine and leucine) and protein efficiency ratio.
Wandersleben et al. (2018)	Lupin grit flour	Bread (5, 10 and 15%)	Increased the protein content and specific volume of the bread with overall acceptable sensory evaluation.

Lu, Brennan, Serventi, & Brennan (2018)	Mushroom powder	Bread (5, 10 and 15%)	Textural, rheological properties and volume of bread decreased.
Coda, Varis, Verni, Rizzello, & Katina (2017)	Faba bean flour	Bread (30%)	Increased protein content and hardness of the bread but decreased volume and protein digestibility. Glycaemic index found to be lowest in bread made with faba bean flour.
Bagdi et al. (2016)	Aleurone-rich flour	Bread (40 and 75%)	Increased protein, fat, ash and fibre content of the bread but reduced loaf volume, texture and appearance.
Previtali et al. (2014)	Lentil flour	Bread (10, 20 and 30%)	Inclusion of lentil flour above 20 and 30% levels reduced texture and sensory properties of bread but increased protein and fibre content of the bread.
Swieca et al., (2013)	Onion skin	Bread (1-4%)	Onion skin decreased the <i>in vitro</i> protein digestibility and bioaccessibility of antioxidant capacity due to the interaction of protein and flavonoids.
Mohammed, Ahmed, & Senge (2012)	Chick pea flour	Bread (10, 20 and 30 %)	Increase water absorption and dough stability but decreased loaf volume and textural properties of the bread.
Lau, Zhou, & Henry (2016)	Butter, coconut oil, grapeseed oil and olive oil	Bread (20%)	Glycaemic response reduced with fat addition due to the amylose lipid complex formation.
Coelho & Salas-Mellado (2015)	Chai flour	Bread (7.8 and 11%)	Increased polyunsaturated fatty acid (PUFA) and ratio of PUFA to saturated fatty acid (SFA) in bread. Protein, lipid, fibre and firmness of the product increased.
Takeungwongtrakul, Benjakul, & H-Kittikun (2015)	Shrimp oil	Bread (1, 3 and 5%)	Improve loaf volume but decreased chewiness, gumminess and resilience properties of bread. Addition up to 3 % had no adverse effect on sensory parameters.
Marpalle, Sonawane, & Arya (2014)	Flaxseed flour	Bread (5, 10 and 15%)	Increased water absorption and stickiness but reduced loaf volume and texture of bread. Bread with 10 % flaxseed flour accepted overall.

2.5 Effect of combining starch, protein and lipid sources

Cereal based food products (pasta and bread) are formed from a variety of micronutrients including protein, lipid and starch. During food processing, these micronutrients undergo different complex interactions which influence physicochemical, nutritional (starch and protein digestibility), sensory, and antioxidant properties of the final product (Parada & Santos, 2016). Shah, Zhang, Hamaker, & Campanella (2011) reported that food formulation with these type of micronutrients play a significant role to alter the functional and nutritional properties of food including texture, flavour and digestibility. Zhang & Hamaker, (2003) showed a three component (starch, protein and lipid) interactions by rapid visco-analyser (RVA). They found that due to the interaction of the starch, whey protein and free fatty acids, the viscosity of the product was significantly changed. Also, Zhang & Hamaker (2004) reported that the starch-lipid and protein-lipid complexes were responsible for the formation of the three dimensional amylose, whey protein and free fatty acids (FFA) complex. In the complex, whey protein formed a bridge between the negatively charged FFA and the positively charged denatured protein molecules by electrostatic interaction and this complex was more stable than the amylose-lipid complex (Zhang & Hamaker 2004). The controlled interaction between starch, protein and lipid have profound effects on rheological properties. Increase in the shear rates during the complex formation resulted in the reduction of viscosity of the product Shah et al. (2011). However, recently, Wang, Zheng, Yu, Wang, & Copeland (2017) showed that maize starch, β -lactoglobulin and fatty acid formed more ordered V-type crystalline structure compared with binary starch-fatty acid complexes. Starchy food and its digestibility is more related to its ingredient composition and interactions. Starch, protein and lipid complexes and their

susceptibility to digestive enzymes is important and with potential implications for human nutrition. Annor, Marcone, Bertoft, & Seetharaman, (2013) explored the effect of ternary (starch-protein-lipid) interactions on the *in vitro* starch digestibility and eGI of kodo millet flour. The authors reported a significant increase in the starch digestibility after removal of protein and/or lipid, particularly after lipid removal. Also, Zheng et al., (2018) investigated the effects of fatty acid chain length and degree of unsaturation on *in vitro* digestibility of starch-protein-lipid complexes. They reported that shorter chain length fatty acids and lower unsaturation formed more unstable ternary complexes which reduced the starch digestibility of the product. Recently, Chen et al., (2018b) examined the structural, physiochemical and *in vitro* starch digestibility properties of swollen maize starch (MS), maize oil (MO) and zein protein (ZP) complex. They reported that ZP and MO formed complexes with MS and restricted the enzymatic hydrolysis. Also, decreased viscosity of the product retarded the mobility of MO and ZP and hindered interaction between MS, MO and ZP.

2.6 Starch digestibility

Starch is a complex carbohydrate stored in plants and utilised in the human diets as a main energy source, it is especially abundant in the cereals and legumes. The World Health Organisation (WHO) recommended a reduction in consumption of free sugar to less than 5% / day in the daily diet, as this would be beneficial for health (Borczak, Sikora, Sikora, Dobosz, & Kapusta-Duch, 2017). Starch can be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistance starch (RS), digestion begins in the mouth and continues in the small intestine. Some of it is broken down into glucose units by salivary α -amylase and further hydrolysis occur by the action of pancreatic amylase in the small intestine (Santos et al., 2012). In many studies, for

the estimation of blood glucose response after consumption of food, the predictive glycaemic response is used (Brennan, Menard, Roudaut, & Brennan, 2012). In this study, an *in vitro* starch digestibility method was used. This method has been validated as well as standardised more than any of the other methods mentioned in Table 2.9. It has been reported that *in vivo* methods were neither time nor cost efficient in the determination of the glycaemic response. The method used in this study was capable of accurately predicting the glycaemic response of a variety of foods. Starch digestibility and glycaemic response are influenced by number of factors to control the sharp increase in blood glucose level including product type (food matrix), processing (e.g. mixing, cooking, extrusion type) and other ingredients in the product (lipids, proteins, polyphenols) (Brennan, Brennan, Derbyshire, & Tiwari, 2011) (Table 2.10). These include starch gelatinisation and the presence of protein, fat and fibre components in the food matrix (Zhang, Sui, & Huang, 2017). The glycaemic response is a tool which is used to predict the food matrix released blood glucose release and absorption. Augustin et al. (2015) reported that foods with a high GI are rapidly digested and absorbed into the blood which in turn resulted in a greater insulin demand. Many researchers showed that high GI diets are associated with outbreak of type 2-diabetes (Bhupathiraju et al., 2014), coronary heart disease (Mirrahimi et al., 2012), breast cancer (Mullie, Koechlin, Boniol, Autier, & Boyle, 2016) and obesity (Schwingshackl & Hoffmann, 2013). It has been suggested that foods having a low glycaemic index are good for health (Table 2.11). Some results have shown a relationship between the low glycaemic index and high protein diet with decrease in the obesity complications (Saris, 2005). Borczak et al. (2017) reported that the consumption of low GI foods may contribute to a reduced risk of cardiovascular disease, diabetes and breast and colorectal cancer.

2.7 *In vitro* methods to determine starch digestibility

At present, many researchers have reported *in vitro* methods for carbohydrate digestibility and mimic the gastrointestinal behaviour of food matrix. These methods are less expensive, more rapid, less labour intensive and no ethical restrictions. Many of the methods differ from each other in terms of the enzyme used, time and the temperature of digestion. Granfeldt et al. (1992) developed a procedure in which food is chewed for 15 s by healthy volunteers prior to treatment with digestive enzymes (pepsin and porcine pancreatin α -amylase) and reported the digestibility of carbohydrate can be improved. Also, Casiraghi, Brighenti, Pellegrini, Leopardi, & Testolin (1993) evaluated starch digestibility and glycaemic response of rice and reported that sample was pre incubated with salivary amylase for 5 min and then treated with pepsin and pancreatin. The effect of the addition of α -amylase on the hydrolysis of starch in wheat bread was studied by Brennan, Blake, Ellis, & Schofield (1996). However, McCleary, MMcNally, & Rossiter (2002) discover a protocol for the determination of digestibility of food matrix and reported that food materials were treated with pancreatic α -amylase and amyloglucosidase in a shaking water bath at 37 °C for 16 h and release of glucose content. Most recently, Chung, Shin, & Lim (2008) developed a procedure for the estimation of starch digestibility and glycaemic index of chemically modified corn starch. The sample digested with porcine pancreatic α -amylase and amyloglucosidase. *In vitro* digestibility of maize and potato starch was determined by the use of porcine α -amylase, pepsin and pancreatin enzymes. Glucose concentration in the supernatant was determined by using a glucose oxidase colorimetric analysis kit (Dhital, Shrestha, & Gidley, 2010). Utrilla-Coello et al. (2014) used pepsin and amyloglucosidase for digestion on banana starch and the digestibility

is expressed in terms of rapidly digested starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and total starch (TS). Table 2.9 depicted different *in vitro* methods were developed over period of time.

Table 2.9 Methods of *in vitro* starch digestibility.

Reference	Enzyme used, pH and temperature			Digestion time
	Oral phase (OP)	Gastric phase (GP)	Intestinal phase (IP)	
Granfeldt et al. (1992)	Chewed by volunteers	Pepsin pH: 1.5, 37 °C	Porcine pancreatin α -amylase pH: 6.9, 37 °C	OP: 15 min GP: 30 min IP: 3 h
Casiraghi et al. (1993)	Salivary amylase	Pepsin pH: 2.0, 37 °C	Pancreatin pH: 6.9, 37 °C	OP: 5 min GP: 1 h IP: 5 h
Brennan et al. (1996)	-	Pepsin pH: 1.5, 37 °C	Porcine pancreatin α -amylase pH: 6.9, 37 °C	GP: 10 min IP: 5 h
(Cleary et al., 2002)	-	Pancreatin α -amylase and amyloglucosidase (pH: 6.0, 37 °C)		16 h
Chung et al. (2008)	-	Porcine α -amylase and amyloglucosidase (pH: 6.0, 37 °C)		3 h
(Dhital et al., 2010)	Porcine α -amylase	Pepsin pH: 2.0, 37 °C	Pancreatin and amyloglucosidase (pH: 6.0, 37 °C)	OP: 20 s GP: 30 min IP: 8 h
(Utrilla-Coello et al., 2014)	-	Pepsin pH: 1.5, 37 °C	Pancreatin and amyloglucosidase (pH: 6.0, 37 °C)	GP: 30 min IP: 3h

Table 2.10 Effect of various food components on starch digestibility and glycaemic index.

Food component used	Year	Findings	Author
Guar gum	1996	Blood glucose level decreased due to the gum acting as a physical barrier to starch digestion and increasing the viscosity of digesta.	Brennan et al. (1996)
Lauric acid, myristic, palmitic and oleic acids	2000	Inhibited enzymic hydrolysis by the formation of an inclusion complex consisting of starch and lipid. It also decreased the solubility and increased the gelatinisation temperature.	Crowe, Seligman, & Copeland (2000)
Beans	2004	Reduction of starch digestibility due to the intact cell structures of beans forming a protective covering to starch granules and reducing starch swelling.	Vargas-Torres et al., (2004)
β -glucan	2007	Cereal β -glucan increased the viscosity of the gastrointestinal tract contents and delayed gastric emptying thereby decreasing postprandial glycaemia and insulin secretion.	Lazaridou & Biliaderis (2007)
Lentil, peas and chickpea	2008	Protein content increase the starch-protein interactions in the food matrix and reduced starch gelatinisation and digestibility.	Chung, Liu, Hoover, Warkentin, & Vandenberg (2008)
Legume	2010	Presence of protein in the food matrix creates a stronger network and reduces the capacity of enzymes to attack the starch granules, thereby delaying starch digestion	Chillo, Monroe, Mishra, & Henry (2010)
Inulin and psyllium	2015	Inulin and psyllium in the food matrix encapsulate the starch granules which reduce the water availability for starch gelatinisation and reduce the accessibility of starch molecules to digestive enzymes. granules	Foschia, Peressini, Sensidoni, Brennan, & Brennan, (2015a)
Olive oil and coconut oil	2016	Lower glycaemic response observed due to the amylose-lipid complex (ALC) formation. Coconut oil contain saturated fatty acids (lauric and myristic acid) which contribute to form ALC.	Lau, Zhou, & Henry, (2016)

The presence of protein and lipid in the diet may reduce health problems such as type 2 diabetes and cardiovascular disease (CVD). It helps to reduce the abdominal fat mass and low density lipoprotein (LDL) cholesterol level which is related to CVD disease (Parker, Noaks, Luscombe, & Clifton, 2002). Replacing cereal with animal protein in food products may preserve lean body mass during weight loss and result in improved insulin glucose uptake in skeletal muscle. Latner & Schwartz (1999) reported that intake of food with high protein helps to reduce weight loss by increasing satiety. Delays in starch hydrolysis may be responsible for the slow glucose release, which can lower insulin levels in the bloodstream. Postprandial blood glucose levels depend on the rate of absorption of glucose into the blood stream and the rate of removal of glucose from circulation by blood tissues. Lipid is known to reduce the glycaemic response through reduced absorption of glucose by reducing starch digestibility due to the formation of an amylose-lipid complex with starch that is resistant to enzymatic digestion (Ai, Hasjim, & Jane, 2013). Blood levels of glucose and insulin after consumption of food vary and are in proportion to the amount of glucose consumed. Glucose produced as a result of starch digestion is absorbed and transported to the liver, any excess production of glucose may be converted into glycogen. A lower glucose response is considered beneficial for human health (Brennan & Cleary, 2005). Fish contains protein and lipid that counteract blood glucose levels after the consumption of food. It affects the glycaemic response by reducing the accessibility to the digestive enzyme and also ability to form complex with starch-protein and protein-lipid.

Table 2.11 Effect of glycaemic index (GI) and human health.

Author	Title	Findings
Chiasson et al. (2002)	Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial	Low GI food inhibits the conversion of starch into glucose which reduced the incidence of type 2 diabetes by 36%.
Nilsson, Stenberg, Frid, & Holst (2004)	Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins	Evidence of a beneficial effect from the consumption of a protein rich diet and capacity to stimulate insulin release. The stimulating effect might be due to the release of specific amino acids during digestion.
Gnagnarella, Gandini, Vecchia, & Maisonneuve (2008)	Glycaemic index, glycaemic load, and cancer risk: a meta-analysis	Showed that low GI foods are beneficial to reduce the cancer risk compared to high GI food.
Ludwig et al. (1999)	High glycaemic index foods, overeating, and obesity	Consumption of low GI diet in adults, children and adolescents showed decreased hunger, increased satiety and decreased food intake.
Burris, Shikany, Rietkerk, & Woolf, (2018)	A low glycaemic index and glycaemic load diet decreases insulin-like growth factor-1 among adults with moderate and severe acne: a short-duration, 2-Week randomized controlled trial	A low GI diet decreased concentration of insulin growth factor-1 (IGF-1) which is responsible for acne pathogenesis.
Evans et al. (2017)	Glycaemic index, glycaemic load, and blood pressure: a systematic review and meta-analysis of randomized controlled trials	A lower glycaemic diet reduced body weight and decreased blood pressure.

It has been previously documented that it is possible to manipulate a product's structure by adding protein and lipid rich ingredients to traditional cereal based foods and to achieve a reduction in starch hydrolysis and, hence, the glycaemic response (Brennan, Derbyshire, Tiwari, & Brennan, 2013a).

2.8 Total phenolic content (TPC) and antioxidant capacity

Phenolic compounds in food products mainly occur in two forms, basic phenolic compounds and polyphenols. They contain a hydroxyl group which is tightly bound to the aromatic ring. Dietary polyphenols are the main source of antioxidants for human use. They exhibit many health beneficial effects especially in the treatment and prevention of cancer (Chen et al., 2011), cardiovascular diseases (Mursu et al., 2008), anti-ulcer (Zakaria et al., 2011), anti-inflammatory (Beara et al., 2012) anti-allergenic (Chung & Champagne, 2009), anti-coagulant and anti-microbial (Silva, Rodrigues, Feás, & Estevinho, 2012) activities. At an alkaline pH the phenolic compound present in the food can be oxidised by oxygen with a side chain group of molecular peptides to quinones and protein crosslinking occurred. These quinones can react with the sulfhydryl (SH) and amino groups of proteins and resulting in the formation of high molecular weight brown colored pigments known as tannins. Tannins are highly reactive and can readily combine with SH and amino groups of proteins. These protein and phenol complexes inhibit the hydrolysis of proteolytic enzymes in the small intestine and decreased the protein digestibility of the food product (Prodpran, Benjakul, & Phatcharat, 2012) (Table 2.13). Free radicals, (superoxide anion, hydroxyl radical and nitric oxide radical) present in the human body are responsible for the occurrence of many harmful diseases including cancer (Kinnula & Crapo, 2004), cardiovascular disease (Rahman, Hosen, Islam, & Shekhar, 2012) and Alzheimer's disease (Vajragupta, Boonchoong, & Wongkrajang, 2000). An antioxidant is molecule which is capable of inhibiting the oxidation of other molecules. Cereals contain phenolic compounds and are a good source of antioxidants which have the potential to scavenge the free radicals and inhibit the lipid peroxidation in the food matrix (Dziki, Rozylo,

Gawlik-Dziki, & Swieca, 2014). Recently, Sayed Ahmad et al. (2018) showed that enrichment of bread with protein (2-6%) increased its phenolic content and antioxidant properties two times compared to control bread. Also, Seczyk, Swieca, & Gawlik-Dziki (2016) reported that pasta fortified with carob flour(1-5%) significantly increased total phenolic content and antioxidant activity. Khan, Yousif, Johnson, & Gamlath (2013) revealed that phenolics and antioxidant activity in cooked pasta decreased compared to the raw uncooked pasta due to the action of oxygen, water, and heat treatment during pasta processing and cooking induce the oxidation of some sensitive phenolics antioxidants. Recently, Nalinanon, Benjakul, Kishimura, & Shahidi (2011) reported that threadfin bream protein hydrolysate made by skipjack tuna pepsin possess functional peptides with antioxidant properties.

2.9 *In vitro* method of TPC and antioxidant capacity

Several methods for estimation of TPC and antioxidant activity have been reported by researchers. Many of the methods differ from each other in terms of the chemicals used, reaction time and the absorbance reading. Singleton, Orthofer, & Lamuela-Ravents (1999) showed that Folin-Ciocalteu was used to measure TPC of complex food products by an oxidation and reduction reaction. Its is based on the single electron transfer (SET) principle in which the electron is transferrd from the phenolic compound in alkaline medium to molybdenum to form blue complex. It is simple and convenient method and also showed correlations with DPPH and oxygen radical absorbace capacity (ORAC) assay (Shahidi, Liyana-Pathirana, & Wall, 2006). However, Edelman, Diewok, Schuster, & Lendl (2001) developed a rapid method of determination of total phenolic content from red wine using infrared spectroscopy. Brand-Williams, Cuvelier, & Berset (1995) invented the 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) method which is based on the

electron and hydrogen transfer principle in which absorption intensity of the sample is decreased by the action of electron transfer and the purple coloured solution changes to pale yellow. Antioxidant of food products has also been determined by Re et al. (1999) using the ABTS assay in which highly stable chromatophoric 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical reduced by antioxidants. Also, ferric reducing antioxidant power (FRAP) method was used to analyse the antioxidant activity of the food material. It works on the ability of the phenolics, present in the sample, to reduce the yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to a blue ferrous complex (Fe(II)-TPTZ) by the action of electron donating antioxidants (Benzie & Strain, 1999). Huang, Ou, Hampsch-Woodill, Flanagan, & Prior (2002) developed the oxygen radical absorbance capacity (ORAC) protocol for the estimation of antioxidants in food products. It uses a fluorescence probe to detect the damage of proxy radicals by the action of antioxidant in the food sample. Kuo, Yeh, & Pan (1999) established a rapid photometric method (Inhibition of linoleic acid peroxidation) (IPO) for antioxidant activity. It is based on inhibition of haemoglobin-catalysed peroxidation of linoleic acid. Table 2.12 shows the phenolic content and antioxidant activity estimation methods.

Table 2.12 TPC and antioxidant estimation methods.

Method name	Chemical used	Absorbance (nm)	Time for incubation
Folin-Ciocalteu Assay (Singleton et al., 1999)	Folin-Ciocalteu reagent, sodium carbonate and gallic acid	760	2 h
DPPH Assay Brand-Williams, Cuvelier, & Berset, (1995)	2,2-Diphenyl-1-picrylhydrazyl (DPPH), trolox, methanol	515	1h
ABTS method Re et al. (1999)	2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid),	734	6 min
FRAP assay Benzie & Strain, (1999)	Gallic acid and ferri-tripyridyltriazine	593	4 min
ORAC (Huang et al., 2002)	Trolox, 2,2'-Azobis-2-amidinopropane- dihydrochloride (AAPH) and fluorescein	485	35 min
IPO Assay Kuo et al. (1999)	Linoleic acid, hemoglobin and hydroperoxyoctadecadienoic acid	480	18min

Table 2.13 Influence of various food components on TPC and antioxidant activity.

Food component used	Year	Findings	Author
Brown algae	2017	<i>In vitro</i> bioaccessibility of antioxidant capacity increased with supplementation of brown algae to the bread due to the protein-phenolic complex formation.	Rozylo, Hameed Hassoon, Gawlik-Dziki, Siastała, & Dziki (2017)
Flaxseed	2017	Supplementation with 5% flaxseed increased phenolic content (93%) and antioxidant activity (176%).	Seczyk, Swieca, Dziki, Anders, & Gawlik-Dziki (2017)
Amaranth flour	2016	TPC and antioxidant capacity of pasta enriched with DAL exhibit reduction due to the cooking of pasta.	Cardenas-Hernandez et al. (2016)
Lentil flour	2015	Marked increase in the phenolic and antioxidant content was observed due to the effect of extrusion which hydrolysis the phenols bound to the fibre and protein.	Morales et al. (2015)
Quinoa leaves	2013	Quinoa leaves in bread increased the phenolic content (2.9 fold) and antioxidant activity (5.1 fold) due to baking heat treatment. It allows the liberation of insoluble bonds in the phenolic compound which increased antioxidant capacity.	Swieca, Seczyk, Gawlik-Dziki, & Dziki (2014)
Bean flour	2010	Phenolic content was increased. Probable reason is cleavage of phenolic compound and release of simple phenolic molecules.	Gallegos-Infante et al., (2010)
Buckwheat flour	2009	<i>In vitro</i> simulated digestion increased the phenolic and antioxidant capacity of bread. Release of phenolic compounds by action of digestive enzyme.	Gawlik-Dziki, Dziki, Baraniak, & Lin, (2009)

2.10 Inclusion of fish protein into cereal products and its effect on *in vitro* protein and starch digestion as well as antioxidant capacity and sensory evaluation

Fish protein powder (FPP) is a dried and stable fish product used for human consumption in which protein is more concentrated (65-90%) than in the original fish flesh (Shaviklo, Thorkelsson, Arason, Kristinsson, & Sveinsdottir, 2010). Due to its strong interaction with other proteins and high gelation ability FPP has been used in the food industry as a binder, dispersing agent and emulsifier in preparing herring roe, fillet blocks and restructured products from beef, pork and chicken (Pires et al., 2012). Colour is also an important quality attribute of fish protein ingredient. The colour of FPP varies from light grey to creamy or pinkish depending on the type of fish used also particle size (Shaviklo et al., 2010). Research has shown that FPP is a valuable protein supplement to improve the protein quality particularly in the diets of preschool children where it may aid in the increase of weight and height of the children (Pee & Bloem, 2009). Vakily, Seto, & Pauly (2012) reported that FPP helped to increase the protein content of the diet and was beneficial for infants and children under five in Sierra Leone. Fish protein powder was better utilised than vegetable protein and increased the content of nutrients in the diet and improved the utilisation of the total diet especially children under five. Friedman (1996) revealed that the nutritive value of cereal protein increased when combined with a FPP. The addition of 3% of FPP to wheat flour having protein content of 10.4% increased its protein content to 12.4% with an increase of net protein utilisation from 50 to 67%. Shaviklo, Olafsdottir, Sveinsdottir, Thorkelsson, & Rafipour (2011) studied quality characteristics and consumer acceptance of a high fish protein puffed corn fish snack by sensory analysis. Puffed corn fish snack products were

prepared by the inclusion of 3%, 5%, 7% and 9% fish protein powder. Snacks with 9% fish protein powder exhibited lower liking in odour, texture, flavour, and overall acceptability than those of 7 %. Seasoning of extruded puffed corn snacks with 7% FPP were liked by Iranian children aged 7-12 years old. Chin, Huda, & Yang (2012) studied the incorporation of different levels (0, 5 %, 10 % and 20 %) of surimi powder as a protein source in noodle. Surimi powder was prepared by oven drying at 60 °C. The noodles showed an increase in the content of protein, fat, lightness, yellowness and cooking yield as the levels of surimi powder increased. However, a significant decreasing trend was observed in carbohydrate, tensile strength, elasticity and hardness. Noodles with 5% FPP incorporated showed significant difference in the colour, hardness and elasticity. Fortification of starchy snacks with fish proteins could be a healthy way to boost nutritional intake and to increase fish protein consumption. However existing research illustrates that the potential uses of fish protein ingredients are affected by functional properties of proteins such as water holding capacity, gelation, foam stability and emulsion capacity Halim, Yusof, & Sarbon (2016). Similarly, Vijaykrishnaraj, Bharath Kumar, & Prabhasankar (2014) evaluated the quality of gluten free pasta prepared with different levels of green mussel powder (2.5%, 5%, 7.5% and 10%). Green mussel powder (5%) blends with chickpea flour revealed 4.5 mg EAA/g extract antioxidant activity, radical scavenging activity of 30 % and reducing power of 25%. Santana, Huda, & Yang (2015) reported that sausage made with different levels of surimi powder 100% and 50% showed texture profile analysis values (hardness, cohesiveness, springiness and chewiness) of 100% Surimi powder were significantly lower. In this case, the water holding capacity and emulsion stability of 100% Surimi powder were lower than the control samples by up to 50%. Pansawat et al. (2008) studied the effects of extrusion

conditions on the physical properties of fish and rice based snacks and concluded that the screw speed and feed moisture affected the product temperature, pressure at die and motor torque and physical properties of rice flour, fish powder and menhaden oil formulation. Extruded snack products with a high expansion ratio and low product density were produced at medium extrusion temperature (135 °C), high screw speed (300 rpm) and low feed moisture (19%). In summary, while there is convincing evidence of the ability to incorporate fish protein into cereal products, there is a need for further research in understanding the effect of incorporation of fish protein and lipid on the physiochemical, nutritional (starch, protein digestibility & antioxidant activity) and sensory properties of the product. Usually, cereal products (pasta and bread) have a low protein content (6-10%) which is also limited in some essential amino acid (Singh, Majumdar, & Venkateshwarlu, 2014). In order to increase the nutritive value of cereal products the incorporation of protein rich fish powder could be important. The level and composition of protein in wheat or semolina flour are of great importance for the cooking and eating qualities of cereal products (Fu, 2008). Adding natural proteins to extruded products can improve the nutritional quality of such foods and maintain a strong dough structure. Extruded products can be divided into two different categories: expanded products, such as snacks and breakfast cereals, made by a high-temperature and short-time process, and pasta products made by a low-temperature process. Fish proteins are more highly digestible than those of plant protein. Digestibility is the hydrolysis of protein by enzymes into amino acids or peptides for absorption in the intestine (Jahan Mihan, 2017). Protein digestibility is the potential parameter to assess the use of protein in the body. Many factors play an important role in digestion and absorption such as phenolic compounds, anti-nutritional factors, protein inhibitors and

processing parameters (Gilani, Xiao, & Cockell, 2012). In extruded food products interaction of protein, starch and dietary fibre diminish the hydrolysis of protein. During the processing of the food product protein molecules experience many changes including lysine residue when high thermal treatment is used and these ultimately affect the overall digestibility of the product. Bhattacharya, Das, & Bose (1988) studied the effect of extrusion process variables (feed ratio, length to diameter ratio, temperature and screw speed) on the *in vitro* protein digestibility of extrudate fortified with wheat flour and minced fish.

Protein digestibility values of extrudates was 86.2% compared to 77.5% in the non-extruded product. The protein digestibility value increased due to the high temperature used of the extrusion process which augmented the inactivation of protease inhibitors present in the wheat flour. Ramya, Prabhasankar, Gowda, & Bhaskar (2015) worked on influence of freeze dried shrimp powder in pasta processing with Indian durum wheat. Pasta was prepared with 2.5%, 5% and 10% concentration level of shrimp meat powder. Protein digestibility of pasta with shrimp meat powder showed a decrease in digestibility compared with control. The control pasta had 93 % protein digestibility whereas shrimp meat fortified pasta (2.5 %, 5 % and 10%) showed 72 %, 90 % 96 % of protein digestibility. (Kadam & Prabhasankar, 2012) studied the effects of shrimp meat incorporation on the cooking, microstructure, texture and sensory characteristics of fortified pasta. Pasta fortified with 10%, 20% and 30% shrimp meat showed an increase in the protein digestibility. Protein digestibility of pasta with wheat flour was 83.99 % and pasta incorporated with shrimp meat (10%, 20% and 30%) exhibited 84.57%, 85.02% and 87.61% respectively. Protein and lipid content of the ingredients play an important role in reducing starch digestibility of the cereal product by creating a barrier

between starch and protein and forming starch-lipid and starch-lipid-protein complexes. Recently, El-Beltagi et al. (2017) studied the impact of incorporation of dried carp fish powder and chickpea flour on pizza protein digestibility. They observed protein digestibility of pizza is increased with inclusion of carp fish powder (5%, 7.50% and 10%) as compared to the chickpea flour made pizza. They reported that protein digestibility increased due to the amino acid present in the carp fish powder protein being highly digestible compared to chickpea protein. Ramya et al. (2015) studied the influence of freeze-dried shrimp meat on pasta starch digestibility and reported that starch digestibility of pasta enriched with freeze dried shrimp meat decreased significantly as compared to the durum wheat semolina pasta. Pasta with freeze dried shrimp meat (2.5%, 5% and 10%) had 60%, 50% and 38% digestibility respectively whereas the control pasta had 72% digestibility. The author elaborated that decreased pasta starch digestibility was due to the shrimp protein entrapping the starch granules in the gluten network and delaying the α -amylase activity.

Currently the food industry utilises synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and propylgallate (PG) avoid lipid peroxidation and that in turn creates a potential health risk. Natural and safe antioxidants have been obtained from fish protein (Chalamaiah, Dinesh Kumar, Hemalatha, & Jyothirmayi, 2012). Vijaykrishnaraj et al. (2014) studied the effect of green mussel powder (GMP) on the gluten free pasta. They reported that pasta with green mussel powder at 2.5%, 5%, 7.5% and 10% showed potential antioxidant effects. A total antioxidant capacity of 8.1 and 11.4 EAA/g of extract was observed in pasta with 5% and 10% green mussel powder respectively. Also, there were significant differences in the scavenging activity of the control and fortified pasta with green mussel powder.

Radical scavenging activity in control sample was 2.51% whereas in pasta fortified with green mussel powder at 5, 7.5 and 10% the radical scavenging activity was 14.30%, 14.60% and 15.20% respectively. This could be due to the degradation of molecules taking place during the gelatinisation of starch molecules of pasta. Cai et al. (2015) worked on the antioxidant properties of protein hydrolysates from the skin of carp fish. They reported that the DDPH radical scavenging activity of fish protein hydrolysates were 83 % (black carp), 72 % (grass carp), 80 % (silver carp) and 88 % (black carp). Nalinanon et al. (2011) studied the antioxidant properties of protein hydrolysates from ornate threadfin bream fish. They found that DDPH radical scavenging activity with 20 % hydrolysis was 8.58 $\mu\text{mol TE/g protein}$ more than 10 % hydrolysis 3.15 $\mu\text{mol TE/g protein}$ and 30 % hydrolysis 6.21 $\mu\text{mol TE/g protein}$. Nazeer & Anila Kulandai (2012) studied the antioxidant capacity of muscle and skin protein hydrolysates of king fish. They reported that radical scavenging activity was observed in muscle and skin of the fish at 37% and 36% respectively. This could be due to the fish protein hydrolysates usually linked with hydrophobic amino acid. Huda, Li Leng, & Xian Yee (2010) studied the effect of fish meat and tapioca flour on the oil absorption and hardness of fish crackers. They reported that protein content of the cracker increased with increased content (10.12% to 23.81%) of the fish to tapioca flour. Linear expansion and oil adsorption of the fish cracker showed decreased value (107-37%) and (39 - 8%) respectively. Hardness of the fish cracker was increased in the high content of fish flesh to the tapioca flour (1312- 2366 N/cm²). However, fish based fortification of products have a negative effect on the sensory parameters of products such as odour and flavour if they are used in the product at inappropriate levels. Chin et al. (2012) studied the effect of different concentrations of fish powder (0%, 5%, 10%, 15% and 20%) on the

preparation of wet yellow noodles. They reported that higher concentration levels resulted in a fishier taste compared to control yellow noodles. Vijaykrishnaraj et al. (2014) researched the effect of different levels of green mussel powder on pasta quality. They observed that inclusion of higher level (7.5% and 10%) in the pasta negatively affected the pasta taste quality. Ramya, Prabhasankar, Gowda & Bhaskar, 2015) also reported that the inclusion of freeze dried shrimp powder affected the sensory characteristics of the pasta product. A higher concentration of shrimp powder used in the pasta resulted in a poor taste and low overall acceptability of the product.

Thus it is possible to use powdered fish material in a range of cereal food products to enhance the nutritional properties of foods as well as maintain the quality characteristics suitable for consumer acceptability. Careful selection of ingredients are required, however, in order to develop such products as ingredient composition, as well as processing parameters, have a significant effect on product quality characteristics. Nevertheless, fortification of foods with powdered fish is a potential mechanism to enhance the amino acid, fatty acid composition of protein poor foods such as cereal based products.

Chapter 3

Materials and methods

Overview

This chapter covers the explanation and information about the materials used, and suppliers for all experimental procedures. An overview of the experimental design is given in Figure 3.1

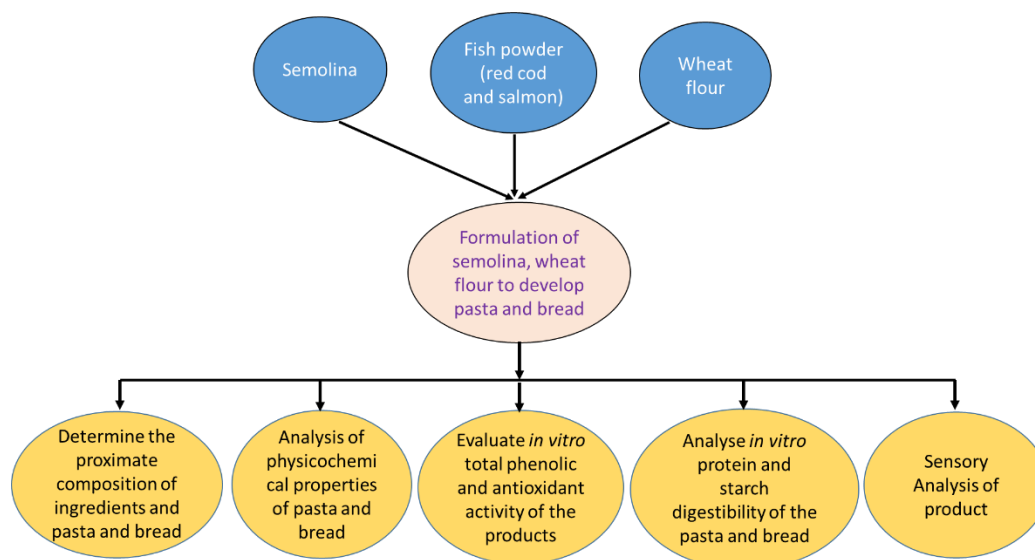


Figure 3.1 Experimental design

3.1 Materials

Red cod fish (*P. bachus*) were bought in an ice condition from Wholesale Seafood Ltd., Christchurch, New Zealand and Salmon fish (*O. tshawytscha*) were also bought in ice condition from Akaroa Salmon Ltd, Christchurch, New Zealand. |Wheat starch (WS) and Wheat Gluten (WG) were purchased from Conservation and Linstrom Suppliers, Christchurch, New Zealand, respectively, while salmon fish oil (SO) cod fish oil (CO) and

coconut oil (CONT) were obtained from Thompsons, Integria Pvt, Ltd. Auckland and New World Supermarket, New Zealand respectively.

3.1.1 Fish powder preparation

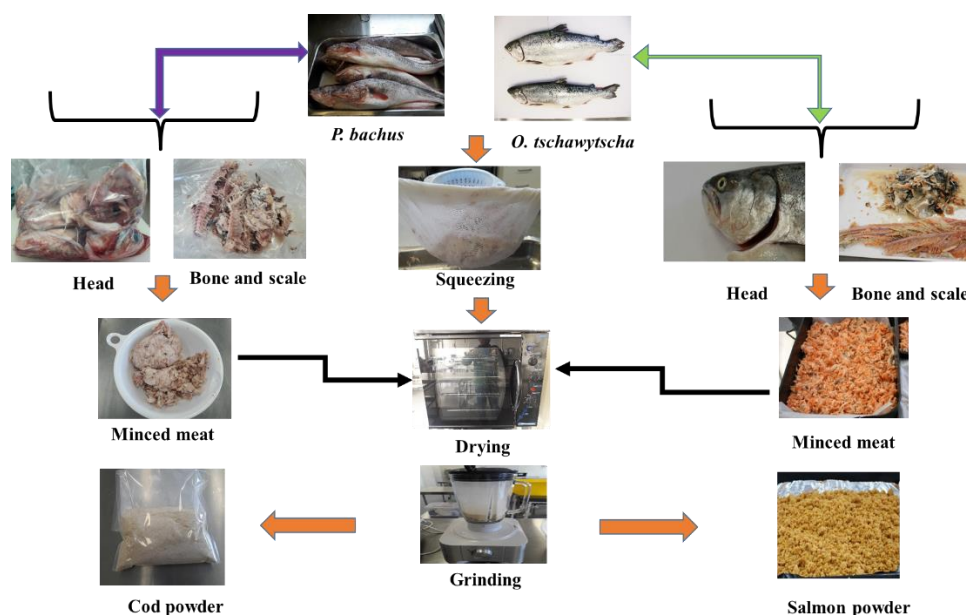


Figure 3.2 Preparation of Fish powder

The red cod and salmon was de-scaled, beheaded, eviscerated and washed with potable water. The dressed fish material was cooked by boiling in water red cod for 10 min and salmon for 5 min. The cooked fish was deskinning and deboned manually before drying in a cabinet dryer (Moffat, E32M, Christchurch, New Zealand) at 45 °C, red cod for 16 h and salmon for 40 h. The dried fish mince was used to produce a powder in a domestic grinder and sieved to pass through a 0.5 mm mesh screen (Majumdar & Singh, 2014; Ortiz et al., 2013). The dried powder was stored in a sealed polythene bag and stored at (-20 °C) temperature until required as shown in Figure 3.2.

3.2 Pasta and bread manufacture

3.2.1 Flour used for pasta and bread

Semolina (Sun Valley Foods, Christchurch, New Zealand) was used for pasta making and wheat flour and wheat starch obtained from a local retail shop, Piko Foods, Christchurch, New Zealand.

3.2.2 Pasta making process

The pasta extrusion was conducted using a Fimar villa verucchio pasta machine Model Number MPF15N235M, (Rimini, Italy) Figure 3.3 and Figure 3.4. The pasta making machine consisted of a stainless steel basin, with an agitator (kneader) (Figure 3.5) to facilitate the appropriate mixing of material. A bronze alloy shaft (screw Figure 3.5) was fitted at the bottom of the basin for conveying material towards the die (Figure 3.6). Cod and salmon powder were used at 0, 10, 15 and 20% (Table 3.1) replacement levels of semolina on a by weight basis. The dry ingredients added to machine and the machine was set on function 1 (mixing), then water was added. Mixing was carried out for 20 min and then the machine was switched to function 2 (extrusion). The long strands of uniform thickness (3.5 mm) were collected on a plastic food tray and cut into lengths of 10-12 cm using a stainless steel knife. For each batch of pasta, 500 g of dry ingredients and 165 mL of water (room temperature) were added to the extruder. The pasta samples were kept in zip lock bags and stored in a freezer at -18 °C until use.

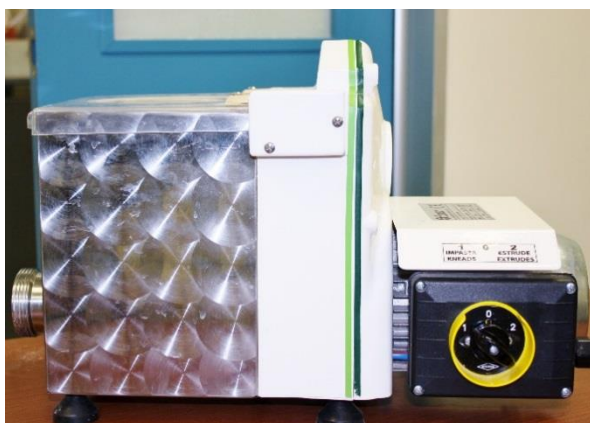


Figure 3.3 Pasta Extruder

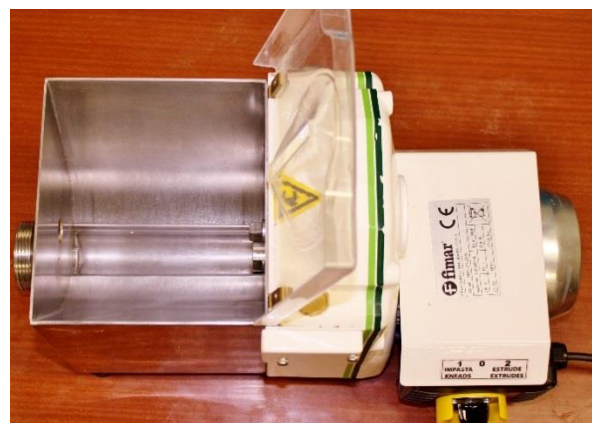


Figure 3.4 Pasta extruder top view

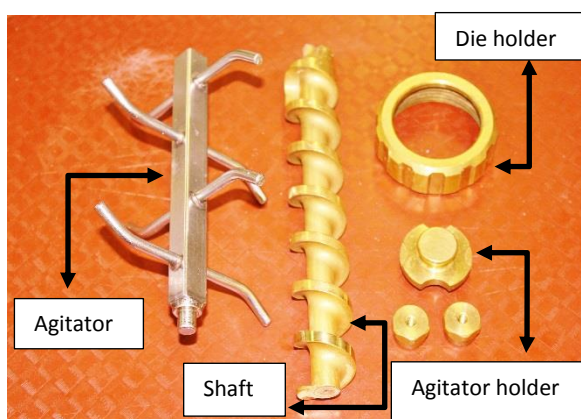


Figure 3.5 Pasta extruder parts



Figure 3.6 Pasta extruder die

Table 3.1 Combination of semolina and fish powder used to prepare pasta the values are given per 100 g.

Sample	Quantity	
	Semolina flour (g)	Cod or salmon fish powder (g)
Semolina Control	100	0
Semolina + 5% fish powder	95	5
Semolina + 10% fish powder	90	10
Semolina + 15% fish powder	85	15
Semolina + 20% fish powder	80	20

3.2.3 Bread making process

The bread ingredients used and formulations are shown in Table 3.2 and 9.1A. The formulation was developed according to (Lin & Zhou, 2018) with some modifications. The bread was prepared by replacing wheat flour with different levels of cod and salmon fish powder (5, 10, and 15% w/w based on wheat flour on a weight basis). The dough was formed by using mixer (Model: BBEK1092, Briscoes, Auckland, New Zealand) to mix for 10 min (Figure 3.7 and 3.8) then it was kneaded by hand for 5 min and rested at room temperature for 5 min. After that dough was moulded (size 9 x 6 x3) (Figure 3.9) into small pieces (50 g each) and proved in an oven (Model: OEB 6.10, Manitowoc, Germany) at 35 °C for 45 min followed by resting in the oven for 15 min (Figure 3.10). The bread was baked at 180 °C in an oven (Model: E32M, Moffat Ltd, Christchurch, New Zealand) for 15 min. After baking, bread was allowed to cool to room temperature for 2 h.



Figure 3.7 Bread dough mixer

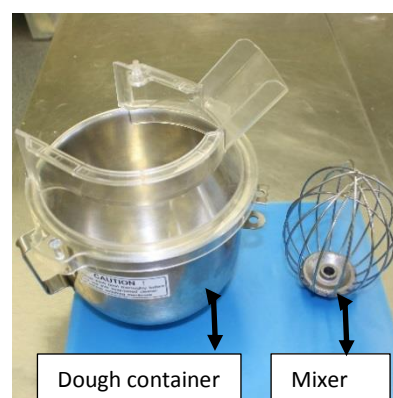


Figure 3.8 Bread mixer parts



Figure 3.9 Bread moulding dish



Figure 3.10 Oven

Table 3.2 Combination of wheat flour and fish powder used to prepare bread the values are given per 100 g.

Sample	Quantity	
	Wheat flour (g)	Cod or salmon fish powder (g)
Wheat flour Control	100	0
Wheat flour + 5% fish powder	95	5
Wheat flour + 10% fish powder	90	10
Wheat flour + 15% fish powder	85	15

3.3 Chemicals, enzymes and buffers used

3.3.1 Chemicals and enzymes required for the protein digestibility analysis :

3.3.1.1 0.1 M HCL solution:

Concentrated (35 %) hydrochloric acid (8.18 mL) MW (HCl) was mixed in 1000 mL RO water.

3.3.1.2 0.1 M NaOH solution:

Sodium hydroxide (4.0 g) was dissolve in 1000 mL RO water.

3.3.1.3 Trypsin, chymotrypsin and protease enzyme:

Pepsin (1031 U/mg), Pancreatin (350 U/mg), Trypsin (2000 U/mg), chymotrypsin (40 U/mg) and protease (5 U/mg) from porcine gastric mucosa, were purchased from Sigma Aldrich, St Louis USA.

3.3.2 Chemical. Buffers and enzyme required for starch digestibility analysis:

3.3.2.1 4 M sodium hydroxide (NaOH) solution:

Sodium hydroxide pellets (32 g) was dissolved in 150 mL RO water. Transfer it to a 200 mL volumetric flask and made to volume up to 200 mL with RO water.

3.3.2.2 1 M HCl solution:

Concentrated hydrochloric acid (16.5 mL) was mixed with 100 mL RO water in a 200 mL volumetric flask. Made to volume up to 200 mL with RO water.

3.3.2.3 0.5 m HCl solution:

Hydrochloric acid (1 mL) was mixed in a 20 mL volumetric flask. Made to volume up to 20 mL with RO water.

3.3.2.4 Sodium bicarbonate (NaHCO₃) solution:

Sodium hydrogen carbonate (42 g) was dissolved in 400 mL RO water. Transferred to a 500 mL volumetric flask and made to volume up to 500 mL with RO water.

3.3.2.5 Ethanol:

Ethanol (99.5 %) was purchased from Sigma Aldrich, St Louis USA.

3.3.2.6 Invertase and Amyloglucohydrolase:

Invertase (300U/mg in 50 % glycerol, stored at -20 °C) and amyloglucohydrolase (3260 u/mg) were purchased from Megazyme Inc. Wicklow, Ireland.

3.3.2.7 Glucose standard solution (5mg/mL):

D-glucose (1g) was dissolved in 150 mL RO water. Transfer to a 200 mL volumetric flask and make volume up to 200 mL with RO water. Store as 5 mL aliquots in freezer at -20 °C

3.3.2.8 Glucose standard solution (10 mg/mL):

D-glucose (2 g) was dissolved in 150 mL RO water. Transfer to a 200 mL volumetric flask and make volume up to 200 mL with RO water. Mix well. Store as 5 mL aliquots in freezer at -20 °C

3.3.2.8 Sodium maleate buffer 0.1 M pH 6:

Maleic acid (11.6 g) was dissolved in 800 mL water then the pH was adjusted to pH 6 using 4 M NaOH. Hydrated calcium chloride, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.3 g) was added to the solution followed by 0.23 g of sodium azide. The volume of the solution adjusted to 1 L with RO water in a volumetric flask.

3.3.2.9 Sodium acetate buffer 0.1 M pH 5.2:

Sodium acetate (13.6 g) trihydrate was added to 900 mL water, the pH was adjusted to pH 5.2 using 0.1 M acetic acid, then 4 mL of 1 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was added and made to volume up to 1 L with RO water.

3.3.2.10 Dinitrosalicylate (DNS) mixture:

DNS (10 g) (3,5-dinitrosalicylic acid) was dissolved in 400 mL of 2 M NaOH at room temperature with vigorous stirring. Then 300 g sodium potassium tartrate tetrahydrate (MW = 282.22 g/mol) was dissolved in 500 mL of distilled H_2O , then these two solutions were mixed then the volume made 1 L using RO water, and the absorbance was read with help of spectrophotometer (VWR, V-1200) at 530 nm. Under alkaline and heating conditions, the reducing sugars contains free aldehyde or keto groups and they can react with DNS to produce 3-amino-5-nitrosalicylate which absorbs light at 530 nm.

3.3.3 Chemicals and buffers required for total phenolic content (TPC) and antioxidant analysis:

3.4.3.1 0.2 N Folin Ciocalteu reagent

Folin Ciocalteu reagent (2 N) was purchased from Sigma Aldrich, St Louis USA. Folin Ciocalteu reagent (2 N) (20 mL) was placed in a 100 mL volumetric flask and made to volume with RO water.

3.3.3.2 7.5 % sodium carbonate (Na_2CO_3)

Sodium carbonate (7.5 g) was dissolved in 100 mL RO water.

3.3.3.3 Gallic acid solution (200 μg)

Gallic acid ($\text{C}_7\text{H}_6\text{O}_5$) (0.040 g) was dissolved in 200 mL volumetric flask with RO water.

3.3.3.4 AAPH (2, 2' azobis (2-amidino-propane) dihydrochloride)

AAPH (0.06456 g) was dissolved into a 10 mL volumetric flask and made up with warmed 37 °C phosphate buffer.

3.3.3.5 Fluorescein ($\text{C}_{20}\text{H}_{12}\text{O}_5$) solution (1mM)

Fluorescein (0.0166 g) was dissolved into 50 mL volumetric flask and filled with phosphate buffer to get 1mM solution.

3.3.3.6 Trolox (6-hydroxy-2, 5, 7, 8 -tetra methylchroman-2-carboxylic acid) (200mM)

A stock solution of 2mM was prepared by dissolving Trolox 0.0250 into 50 mL volumetric flask and made up with phosphate buffer. From stock solution 1 mL was added into a 10 mL volumetric flask and filled with phosphate buffer and made 200 μM Trolox solution.

3.3.3.7 Phosphate buffer (75mM)

Sodium dihydrogen orthophosphate hydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) (1.7234g) and di-sodium hydrogen orthophosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) (17.805 g) were dissolved in 100

mL and 500 mL RO water respectively. In 500 mL volumetric flask, mixed 95 mL ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) and 405 mL ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) and made to volume with RO water.

3.4 Methods

3.4.1 Protein content

The protein contents of the fish powder, raw flours, cooked, uncooked pasta and oven baked bread samples were determined using the Dumas method (Maehre et al., 2018). Samples (600 mg) were weighed in triplicate and loaded individually into the Dumas machine to measure the total nitrogen. The nitrogen content was determined using an Elemental analyser Model Vario MAX CN Hanau, Germany. The instrument works according to the principle of catalytic tube combustion under oxygen and at a high temperature. The combustion gases are separated from the foreign gases. Carbon and N were then separated from each other by specific adsorption columns and then detected in succession using a thermal conductivity detector. The carrier gas was helium. The protein percentage (dry basis) was calculated by the following formula:

$$\% \text{ protein} = \text{N} \times 6.25$$

Where, 6.25 and 5.94 are the correction factor used to convert the nitrogen content of pasta and bread into protein content (Leser, 2013)

3.4.2 Moisture content:

The moisture content was determined using method given by Approved Methods of the AACC (2010). A clean coded aluminium cup was first dried in an oven at 105 °C for 30 min, cooled in a desiccator and weighed. A ground sample (5 g) was placed in the cup and placed in an oven at 105 °C overnight. The cup was allowed to cool in a desiccator.

The weight of the cup plus contents after drying was recorded and moisture content calculated according to the equation below:

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of the dried sample}}{\text{Weight of the sample}} \times 100$$

3.4.3 Fat:

Crude fat was determined using a BUCHI Soxhlet Extraction Unit E-816HE (Luque de Castro & Priego-Capote, 2010). The samples (1 g) were weighed into separate thimbles to perform the extraction. Petroleum ether was then added to the glass tubes and the thimbles were suspended in the glass tube with a holder. The principle is that a dried ground sample is extracted with petroleum spirit; this dissolves fats, oils, pigments and other fat soluble substances. The petroleum spirit was then evaporated from the fat solution by boiling the solvent. After one hour the glass tubes were placed in a hot air oven (105 °C) for 20 mins. The samples were then cooled for 10 min before weighing. The resulting residue was weighed and referred to as either extract or crude fat.

3.4.4 Carbohydrate and energy content

The proximate total carbohydrate content was estimated by subtracting the total fat content, protein content, ash and moisture content from 100 %. The energy value was calculated using the formula described by (Schakel *et al.* 1997)

$$\text{Energy value (Kcal/100 g)} = 4 \times \text{protein (\%)} + 9 \times \text{lipid (\%)} + 4 \times \text{carbohydrate (\%)}$$

3.4.5 *In vitro* protein digestibility

Each sample was analysed for protein digestibility following the method reported previously by (Hsu & Vavak, 1977). The multi-enzyme technique described was used for the determination of *in vitro* protein digestibility of cooked pasta and bread sample. A 50 mL aliquot of protein suspension was prepared in distilled water (6.25 mg

of protein/mL), adjusted to pH 8 with a solution of 0.1 N HCL and /or 0.1 N NaOH, and placed on magnetic heating stirring block at 37 °C. The multi-enzyme solution (1.6 mg/mL trypsin, 3.1 mg/mL chymotrypsin and 1.3 mg/mL peptidase) was maintained in an ice bath and adjusted to pH 8.0 with 0.1 N HCL and /or 0.1 N NaOH. Five mL of the multi-enzyme solution was then added to the protein suspension, which was maintained at 37 °C. The decrease in pH was measured after the addition of an enzymatic solution at every min for 10 min using a digital pH meter (S20 Seven Easy™, Mettler Toledo, USA). The percent protein digestibility (Y) was calculated by using following equation:

$$Y = 210.46 - 18.10 X,$$

Where X represents the change in pH after 10 min.

3.4.6 *In vitro* starch digestibility and glycaemic response

The *in-vitro* digestion method adopted by (Foschia *et al.*, 2015b) was used to evaluate carbohydrate digestibility of the pasta and bread samples. This method is relatively quick and less expensive compared to other *in vivo* and *in vitro* methods while still having an acceptable level of accuracy. This method measures the amount of free reducing sugars released during the enzymatic hydrolysis. Pasta was cooked in boiling tap water (600 mL) according to optimum cooking time and cut with knife in order to obtain a 2-5 mm size. The samples (2.5 g) were suspended in 30 mL of RO water and placed on a pre-heated 15 place magnetic heated stirring block (IKAAG RT 15, IKA-WERKE GmbH & Co., Staufen, Germany) and held at 37 °C with constant stirring. Stomach digestion was initiated by adding 0.8 mL 1M HCl and 1 mL of 10 % pepsin (Sigma Aldrich, USA) solution in 0.05 M HCl with continued stirring and incubated at 37 °C for 30 min. One millilitre of aliquots were taken (time 0) and added to 4 mL ethanol.

Amyloglucosidase (0.1 mL) was added to the digestion pot in order to prevent end product inhibition of pancreatic α -amylase. Small intestine digestion was mimicked by the addition of enzyme solution (5 mL of 2.5% Pancreatin (Sigma Aldrich, USA) solution in 0.1 M sodium maleate buffer pH 6) with constant stirring at 37 °C for 120 min and aliquots withdrawn after 20, 60 and 120 min and added to 4 mL ethanol. The samples were stored at 4 °C until analysis of reducing sugar content using the 3,5-dinitrosalicylic acid (DNS). For the measurement of the reducing sugars, all test tubes containing the sample aliquots were centrifuged at 1000 g for 10 min. Clean, dry glass test tubes were placed in a stainless steel test tube stand, then 0.05 mL of a sample aliquot from each replicate was placed in individual glass test tubes. A 0.05 mL reagent blank (RO water), 0.05 mL of 5 mg/mL glucose standard and 0.05 mL 10 mg/mL were placed in separate tubes. Then, 0.25 mL of enzyme solution (1 % Invertase and 1% amyloglucosidase) was added to each glass tube and all the tubes were kept for 20 min at room temperature before 0.75 mL of the DNS (reagent) was added to each tube, the tubes were covered and heated for 10 min in a boiling water bath. The glass tubes were then cooled before adding 4 mL of RO water and the absorbance was read at 530 nm. The spectrophotometer was adjusted to zero using a RO water blank. Reducing sugar release was calculated as mg /g sample and plotted against time and area under the curve (AUC) was calculated by dividing the graph into trapezoids.

3.4.7 Amino acid analysis of pasta and bread

To hydrolyse the protein within the sample into its constituent amino acids, the sample was acid digested with 6 N hydrochloric acid in an oven at 110 °C for 20 h. The amino acids in the samples were then determined using an Agilent 1100 series (Agilent

Technologies, Walbronn, Germany) high-performance liquid chromatography following the methodology proposed by (Heems, Luck, Fraudeau, & Verette, 1998). The extracted amino acid samples were injected into HPLC equipped with a 150 × 4.6 mm, C18, 3u ACE-111-1546, (Winlab, Scotland, UK) column for amino acid separation. Column flow rate was 0.7 mL/min and the temperature was kept at 40 °C. O-phthaldialdehyde (OPA) was used as a fluorescence derivative reagent for primary amino acids, and 9-fluorenylmethyl chloroformate (FMOC) for secondary amino acids. Detection utilised a fluorescence detector with an excitation of 335 nm and emission of 440 nm for primary amino acids. At 22 min, the detector was switched to excitation 260 nm and emission at 315 nm to detect secondary amino acids such as proline. The amino acid results are expressed in mg amino acids per g protein of sample.

3.4.8 Digestible indispensable amino acid score (DIAAAS)

For the assessment of protein quality in the pasta enriched with SFP samples, DIAAS values were calculated using true ileal amino acid digestibility of individual dietary (IAA) against two amino acid scoring pattern of the reference protein; child (6 month to 3 years), and older child (above 3 years), adolescent, adult as recommended by (Gilani, Tome, Moughan, & Burlingame, 2012). Table 7 indicates the true ileal amino acid digestible coefficients (%) for IAAs of salmon and semolina that were used to estimate digestible dietary content of each IAAs in the enriched SFP made pasta sample and the control pasta (Gilani et al., 2012). However, due to the unavailability of amino acid digestibility data, true ileal amino acid digestibility of sulphur amino acids, tryptophan, and tyrosine of fish proteins was assumed to be equivalent to the true ileal crude protein digestibility data. True ileal digestibility coefficients for salmon and semolina

were obtained from a study in humans as reported by a sub-committee of the 2011 FAO consultation on protein quality evaluation in human nutrition (Gilani et al., 2012). To calculate DIAAS of the pasta samples, the digestible IAA reference ratio was calculated for each IAA according to the following equation:

$$\text{IAA reference ratio} = \frac{\text{mg of digestible IAA in 1 g protein of food}}{\text{mg of the same dietary IAA in 1 g of the reference protein}}$$

For a given reference protein amino acid score pattern. DIAAS (expressed in %) was calculated by the following equation (Wolfe, Rutherfurd, Kim, & Moughan, 2016).

$$\text{DIAAS (\%)} = 100 \times \frac{\text{mg of digestible dietary IAA in 1 g of the dietary protein}}{\text{mg of the same amino acid in 1 g of the reference protein}}$$

3.4.9 Fatty acid analysis of pasta and bread

Lipids from pasta and bread samples (1.0 g) were extracted with heptane solvent and analysed in a gas chromatography coupled with a flame ionisation detector (FID) (Perkin Elmer, Waltham, MA, USA). The fatty acid methyl esters were separated in a VARIAN gas chromatograph model CP 7420 equipped with FID and CP-Sil (100m long, 0.25 mm internal diameter and 0.2 um film thickness) fused silica capillary column. One microlitre of the sample was injected and the temperature of the injector and the detector temperature were both 250 °C. The column temperature started at 45 °C with a ramp of 13 °C /min and 4 °C /min until 175 °C and 215 °C respectively which were held for 27 min and 35 min respectively. When the oven temperature reached at 250 °C it was held for 5 min. The fatty acid content was expressed as a percentage of the total fatty acids detected. The standard solution 68 D was used to establish the correction factors for each of the certified fatty acids, which were used to transform the percentage peaks by weight (mg/g of total fatty acids). The methyl ester were quantified through the

integration of the peak area using the software star 6.0. Helium gas was utilised as the carrier gas with a flow rate of 16.7 cm/s (Palmquist & Jenkins, 2003).

3.4.10 Total phenolic content (TPC) and antioxidant capacity

3.4.10.1 Total phenolic content (TPC)

The total phenolic content of supernatant obtained from the *in vitro* gastro-intestinal digestion was measured using the Folin-Ciocalteu method as described by (Li et al., 2007). Freshly prepared 2.5 mL of 0.2 N Folin Ciocalteu reagent and 7.5% Na₂CO₃ was added to the digesta aliquots (0.5 mL) and incubated for 2 h in the dark. The absorbance of the reaction mixture was measured at 760 nm using the V-1200 model (Schimadzu, Maryland , USA). Gallic acid was used as a standard to determine total phenolic content of the samples as mg of Gallic acid equivalents (GAE)/g sample.

3.4.10.2 ORAC (oxygen radical absorbance capacity)

ORAC (oxygen radical absorbance capacity) was determined as described by (Hossain et al., 2017). Briefly, 25 µL diluted extract samples and 150 µL of 10 nM fluorescein were pipetted into each working well of the microplate and incubated at 37 °C for 30 min. Next, 25 µL freshly prepared AAPH solution was added to the pre-incubated microplate. Fluorescence was measured (excitation 544 nm; and emission 590 nm) from the bottom every 60 s for a total of 60 min. A 96 well microplate reader (FLUOstar Omega, BMG LABTECH, Germany) was used for all measurements. Trolox was used as a standard and antioxidant capacity was expressed as mmol Trolox equivalent (TE)/g sample calculated using Omega MARS data analysis software (program version 3.02 R2).

3.4.10.3 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The antioxidant capacity of the samples was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by (Hossain et al., 2017). Briefly, 0.5 mL of crude

extract was mixed with freshly prepared 1 mL of 0.1mM methanolic DPPH (CAS: 1898-66-4, Sigma-Aldrich, St. Louise, MO, USA) solution and incubated in the dark at room temperature for 30 min. The reaction mixture absorbance was measured at 517 nm. In order to calculate the DPPH radical scavenging capacity, trolox (CAS: 53188-07-1, ACROS Organics™, Morris, NJ, USA) was used as a standard and results were expressed as μmol trolox equivalent (TE) per g sample.

3.4.11 Physical characteristics of pasta

3.4.11.1 Optimal cooking time

Pasta samples were defrosted at room temperature (25°C) for 10 min prior to the determination of optimal cooking time. Pasta strands (20 g) were cut into equal lengths of 40 mm and cooked in 300 mL of boiling water. During cooking the optimal cooking time was evaluated by taking a sample strand of pasta every 30 s and observing the time of disappearance of the core of pasta, by squeezing it between two transparent glass side, according to the AACC approved method 66-50 AACC (2010). The time at which the core completely disappeared was taken as the optimal cooking time.

3.4.11.2 Cooking loss

Ten grams of pasta were cooked in 600mL of boiling water for optimal cooking time, rinsed with 100 mL of cold water, strained for 30 s to determine the cooking loss. An aluminium vessel was used to collect the cooking water. The vessel placed in an air oven at 105 °C and evaporated until a constant weight was reached. The residue was weighed and reported as a percentage of starting material.

3.4.11.3 Swelling index and water absorption index

Ten grams of pasta were cooked in 600 mL of boiling water at optimal cooking time, rinsed with 100 mL of cold water, strained for 30 s to determine the swelling index of

the pasta samples. The cooked pasta sample was weighed after cooking and dried at 105 °C until constant weight was reached for estimation of water absorption index.

$$\text{Swelling index (SI)} = \frac{\text{Weight of cooked pasta (g)}}{\text{Weight of pasta after drying (g)}}$$

$$\text{Water absorption index (WAI)} = \frac{\text{Weight of cooked pasta} - \text{Weight of uncooked pasta}}{\text{Weight of uncooked pasta}} \times 100$$

3.4.11.4 Colour measurement

The colour of the pasta and bread samples were measured in terms of L^* (brightness), a^* (redness) and b^* (yellowness) by using a tristimulus colour analyser (Minolta Chroma Meter CR 210, Minolta Camera Co., Japan). The instrument was calibrated using a standard white tile ($L^*=98.03$, $a^*=-0.23$, $b^*=0.25$). The change in colour was determined by calculating the colour differential index (ΔE) using following equation

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Where, ΔL : $L^*_{\text{sample}} - L^*_{\text{control}}$; Δa : $a^*_{\text{sample}} - a^*_{\text{control}}$; and Δb : $b^*_{\text{sample}} - b^*_{\text{control}}$

3.4.11.5 Texture analysis of pasta

The firmness and tensile strength of pasta were evaluated by (Foschia *et al.*, 2015a) Texture Analyser (TA.XT2; Stable Micro Systems, Godalming, UK) equipped with a 5 kg load cell. Prior to the testing of firmness and tensile strength pasta samples were cooked for the optimum cooking time and kept at room temperature for 10 min. The analyser was set at a pretest speed 2 mm/s and test speed 3 mm/s. Samples were loaded onto the platform in groups of five pasta strands together. The probe (blade) was fixed at the top of the samples and cutting force was calculated by lowering the blade. Resistance to uniaxial extension of the cooked pasta was determined by tension test using the A/SPR spaghetti/noodle rig (settings: pre-test speed, 3 mm/s; test speed, 3 mm/s; post-test speed, 5 mm/s; initial distance, 10 mm; final distance, 100 mm;

trigger type, auto 5 g; rate for data acquisition, 200 pps). The results are expressed as maximal breaking strength (N).

3.4.11.6 Volume and Texture analysis of bread

Bread volume was determined by the rapeseed displacement method, following the method (AACC, 2010). The specific volume of bread was obtained through dividing bread volume (mL) by bread weight (g). Texture analysis of the bread was determined using a texture analyser (TA.XT2, Stable Micro Systems, Godalming, UK) equipped with a 25 mm diameter cylinder probe. Bread was cut into slices of 25 mm thickness, which were then used for analysis. The bread samples were compressed twice by probe to provide insight into how samples behave when chewed. The following texture profile analysis (TPA) parameters were automatically recorded by Exponent software: hardness (the peak force of the first compression), springiness (the distance of the detected height during the second compression divided by the original compression distance), cohesiveness (the area of the second compression divided by the area of the first compression), gumminess (hardness \times cohesiveness), chewiness (gumminess \times springiness), resilience (by dividing the upstroke energy of the first compression by the down stroke energy of the first compression). The test settings were as follows: pretest speed: 1.0 mm/s; test speed: 1.7 mm/s; post-test speed: 10.0 mm/s; strain: 40 %; trigger force: 5 g (Liu et al., 2017b).

3.4.12 Sensory analysis

Sensory evaluation was performed on cooked pasta by an untrained a consumer panel. Before testing, all participants were screened for possible food allergies to wheat and fish powder. Ethical approval for the study was granted by the Lincoln University Human Ethics Committee (Appendix A.1). Fifty two panel members were staff and students of

the Lincoln University and recruited via email. Control pasta and pasta samples (25 g) enriched with cod and salmon fish powder were cooked for the optimal cooking time, drained, and kept warm until serving. Panellists were assessed pasta samples for consumer acceptability. Cooked pasta samples were placed in plastic cups/plates, labelled with random 3 digit code and presented to the panellists. The cooked pasta samples were evaluated for the appearance, colour, aroma, taste, texture (in mouth) and overall acceptability (Appendix A.2). Sensory attributes were assessed using the 9-point hedonic scale and the values ranged from 1 to 9, wherein: (1) dislike extremely, (2) dislike very much, (3) dislike moderately, (4) dislike slightly, (5) neither like or dislike, (6) like slightly, (7) like moderately, (8) like very much, and (9) like extremely (Khan et al., 2014) (Appendix A.3).

3.4.13 Rapid visco analyser

The wheat starch (3.0 g) with or without (control sample) added cod oil, salmon oil, coconut oil (15 % of weight of starch) and wheat gluten (10 % of weight of starch) were weighed and added the canister and distilled water was added to obtain 28 g sample weight. The thermal-visco profiles of the resulting pastes were measured by a Rapid Visco Analyser (Perten Instruments, Hagersten, Sweden). Briefly, a programme heating and cooling cycle standard profile 1 was used where the samples were held at 50 °C and heated to 95 °C at 6 °C / min, a holding phase at 95 °C for 1.5 min, a cooling step from 95 to 50 °C at 6 °C / min and holding phase at 50 °C for 2 min. The peak viscosity (PV), trough viscosity (TV), final viscosity (FV) and breakdown (BD) of materials were recorded (Annor et al., 2015).

3.4.14 Complex index (CI)

The complexing index (CI) of samples removed from RVA canister were measured by the method of (Annor, Marcone, Corredig, Bertoft, & Seetharaman, 2015) with modifications as follows. Starch paste (5.0 g) was mixed with 25 mL of distilled water in a 50 mL test tube. The tube was vortexed for 2 min, and 100 µl of the resulting dispersion was mixed with 15 mL of distilled water, followed by the addition of 2 mL of iodine solution (2.0% KI and 1.3% of I₂ in distilled water). The absorbance at 690 nm was measured. Pastes that contained only starch were used as a reference. The complexing index was calculated as follows:

$$CI = \frac{\text{Absorbance of Reference Sample} - \text{Absorbance of Sample}}{\text{Absorbance of Sample}} \times 100$$

3.4.15 Scanning electron microscopy (SEM)

Freeze dried samples were mounted on aluminium pan using doubled sided sticky carbon tape and coated with a thin film of gold (10nm) in a vacuum evaporator. Morphology of WS, WG and TCMs samples were observed by using a scanning electron microscope (SEM) (ZEISS EVO18, Germany) using an accelerating voltage of 10 Kv.

3.4.16 Fourier transform infrared (FT-IR) spectroscopy

The FTIR spectra of freeze dried samples from RVA were obtained using a Vector 33 FT-IR spectrophotometer (Bruker, Ettlingen, Germany) equipped with a KBr beamsplitter and a DLaTGS detector. The spectra will be scanned at room temperature in the range of 4000-400 cm⁻¹ with an accumulation of 64 scans and a resolution of 4 cm⁻¹.

3.4.17 Laser confocal micro-Raman (LCM-Raman) spectroscopy

The freeze-dried samples after RVA measurement were analysed for LCM-Raman spectra by using a Renishaw Invia Raman microscope system (Gloucestershire, United

Kingdom) operated with a Leica microscope (Wetzlar, Germany). A 785 nm green diode laser source was used, and spectra were taken in the range of 3200 to 100 cm^{-1} , with a 7 cm^{-1} resolution approximately. The full width at half height (FWHH) of the peak at 480 cm^{-1} was obtained using Wire 2.0 software.

3.4.18 Differential scanning calorimeter (DSC)

Thermal properties of samples were investigated using differential scanning calorimeter (DSC) (DSC-8000, Perkin Elmer, USA). Sample (2-3 mg) with deionised water (70% moisture content) were scanned from 30 to 110 oC in a sealed aluminium container at a rate of 10 oC/min. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy change (ΔH) were obtained from each analysis with Pyris software (PerkinElmer).

3.4.19 ^{13}C nuclear magnetic response (NMR) spectroscopy

^{13}C NMR spectra of mixtures (starch-oil-gluten) in an aqueous solution (40 mg/mL) with or without the addition of SO, CO, CONT and SA (15%) and wheat gluten (10%) were obtained after acquiring 2500 scans at 25 oC following the method of (Ai, Hasjim, & Jane, 2013).

3.4.20 Statistical analysis

All experiments were performed in triplicate unless otherwise stated. Results were subjected to one way analysis of variance (ANOVA) and significance differences were evaluated by Tukey's comparison test ($P < 0.05$). Statistical software version 17 (Minitab, Australia) was used to perform the statistical analysis of the data.

Chapter 4

The effect of semolina replacement with protein powder from cod fish (*Pseudophycis bachus*) on the physicochemical characteristics of pasta

This chapter is published as:

Ajay Desai., Margaret A Brennan., & Charles S Brennan (2018). The effect of semolina replacement with protein powder from fish (*Pseudophycis bachus*) on the physicochemical characteristics of pasta. **LWT-Food Science and Technology**, 89, 52-57.

Abstract

This study replaced semolina with red cod (*P. bachus*) fish powder in pasta at 5, 10, 15 and 20 g/100 g levels. The effects on the chemical composition, physical properties (optimal cooking time, cooking loss, water absorption index, swelling index and colour) and textural properties (firmness and extensibility) of the supplemented pasta samples were evaluated compared with a control sample. Fortification with fish powder increased protein, lipid and ash contents significantly ($P < 0.05$). Cooking loss increased ($P < 0.05$) with increasing levels of fish powder. However, all pasta samples were in the acceptable range (8 g/100g) for cooking loss. Fish powder incorporation decreased optimal cooking time, swelling index and water absorption significantly ($P < 0.05$), whilst increasing firmness and resistance to uniaxial extension of pasta. The addition of fish powder increased yellowness (b^*) of the pasta significantly ($P < 0.05$) compared to control sample. Thus, pasta fortified with fish powder has the potential to be a technological alternative for the food industry to provide nutritional enriched pasta.

4.1 Introduction

Pasta is the second most consumed food item in the world, and consumption of pasta increased by 2 million tonne in 2014 in relation to 2013 (International Pasta Organisation Survey, 2015). Pasta is a popular food product because of its' versatility, low cost, ease of preparation and nutritional quality (Foschia et al., 2015a). Pasta is a healthy food which contains protein, vitamins and is an important source of carbohydrates with virtually no fat (Foschia et al., 2015b; Krishnan, Menon, Padmaja, Sajeev, & Moorthy, 2012; Sobota, Rzedzicki, Zarzycki, & Kuzawińska, 2015). Cooking quality is the most important consumer attribute of pasta, including parameters such as cooking time, cooking loss, water absorption index, swelling index, texture (Ficco et al., 2016; Gelencser, Gal, Hodsagi, & Salgo, 2008; Sobota et al., 2015). The quality of pasta, and cooking characteristics, are dependent upon the protein-starch network of the pasta product (El-Khayat, Samaan, Manthey, Fuller, & Brennan, 2006). Pasta firmness, elasticity and cooking loss can be related to protein content as well as the starch composition (Samaan, El-Khayat, Manthey, Fuller, & Brennan, 2006). Raw material composition used for the preparation of pasta product affects the physical, chemical and textural properties of pasta (Brennan et al., 2013a; Lu, Brennan, Serventi, Mason, & Brennan, 2016). Currently, there are many studies focused on increasing nutritional value in terms of the protein content of pasta products (de la Peña & Manthey, 2014; Fuad & Prabhasankar, 2012; Padalino, Mastromatteo, De Vita, Maria Ficco, & Del Nobile, 2013). Over the past few decades, wheat pasta has been prepared incorporating different ingredients including, bean flour (Gallegos-Infante et al., 2010) pea flour (Wojtowicz & Moscicki, 2014), shrimp meat (Ramya, Prabhasankar, Gowda, Modi, & Bhaskar, 2015), shrimp mince (Kadam & Prabhasankar, 2012), beef meat (Liu

et al., 2016), green mussel (Vijaykrishnaraj et al., 2014), tomato matrix or bran extracts (Pasqualone et al., 2016) and artichoke extracts (Pasqualone et al., 2017).

Fish powder is a product of fish processing and represents a cheap source of high quality nutrients that can be utilised in the human diet, this is mainly due to high levels of essential amino acids and polyunsaturated fatty acids especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Oliveira, Lourencgo, Sousa, Peixoto Joele, & Ribeiro, 2015; Stevanato et al., 2010). Fish protein has the potential to have beneficial health effects such as manipulation of obesity, hypertension and cardiovascular disease in human beings (Kadam & Prabhasankar, 2010). Fish powder is also a good source of various vitamins (A, D, B6 and B12) and minerals (iron, zinc, iodine, selenium, potassium and sodium) (Anbudhasan & Surendraraj, 2014). Previous studies evaluated the nutritional and physicochemical characteristics of pasta manufactured with fish powder of *Penaeus monodon* (Kadam & Prabhasankar, 2012), *Nemipterus Japonicus* (Chin et al., 2012), green mussel (*Perna canaliculus*) powder (Vijaykrishnaraj et al., 2014) *Oreochromis niloticus* (Monteiro et al., 2016) and pasta enriched with mince of *Catla Catla* mince (Lakshmi Devi, Aparna, & Kalpana, 2013). However, nutritional composition and chemical stability varies depending on the fish species that is utilised and the processing parameters subsequently employed (Schneedorferová, Tomčala, & Valterová, 2015). The physiochemical properties of pasta enriched with partial replacement of semolina wheat flour by red cod powder (*Pseudophycis bachus*) are still unknown. Therefore, the aim of this project was to develop pasta with improved nutritional properties by substituting semolina flour with cod powder at various concentrations and study the changes in nutritional, cooking, colour and textural characteristics of the pasta.

4.2 Materials and methods

4.2.1 Raw materials

Described in section 3.1

4.2.2 Fish powder preparation

Described in section 3.1.1

4.2.3 Pasta production

Described in 3.2.2 section

4.2.4 Proximate chemical composition analysis of pasta

Described in section from 3.4.1 to 3.4.4.

4.2.5 Physical properties

4.2.5.1 Optimal cooking time

Described in section 3.4.13.1

4.2.5.2 Cooking loss

Described in section 3.4.13.2

4.2.5.3 Swelling index and water absorption index

Described in section 3.4.13.3

4.2.5.4 Moisture content

Described in section 3.4.2

4.2.5.5 Colour measurements

Described in section 3.4.13.4

4.2.6 Textural characteristics

Described in section 3.4.11.5

4.2.7 Statistical analysis

Described in section 3.4.22

4.3 Results and discussion

4.3.1 Chemical composition

The proximate composition of the fish powder enriched cooked and uncooked pasta as well as chemical data of raw materials were presented in Table 4.1.

Table 4.1 Chemical composition (%) (on an as-is basis) and energy value (Kcal/100g) of cooked and uncooked pasta fortified with different fish powder levels.

Sample	Parameters Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Moisture (g/100g)	Carbohydrate (g/100g)	Energy (Kcal/100g)
Fish powder	88.75 ± 0.00	1.37 ± 0.02	5.94 ± 0.08	7.92 ± 0.07	-	367.33 ± 1.75
Semolina	12.7 ± 0.05	1.0 ± 0.01	1.1 ± 0.02	10 ± 0.10	72.8 ± 0.24	350.8 ± 2.56
Cooked pasta						
CO	12.63 ± 0.17 ^a	ND	0.46 ± 0.02 ^a	67.90 ± 0.91 ^a	19.01 ± 1.06 ^a	126.55 ± 3.59 ^a
CFP5	16.52 ± 0.29 ^b	ND	0.65 ± 0.01 ^b	65.70 ± 0.98 ^{ab}	17.13 ± 1.24 ^a	134.59 ± 3.97 ^{ab}
CFP10	20.69 ± 0.11 ^c	ND	0.76 ± 0.00 ^c	65.91 ± 0.62 ^{ab}	12.64 ± 0.52 ^b	133.31 ± 2.45 ^{ab}
CFP15	25.15 ± 0.25 ^d	ND	0.89 ± 0.04 ^d	65.74 ± 0.74 ^{ab}	8.22 ± 0.83 ^c	133.46 ± 2.91 ^{ab}
CFP20	29.82 ± 0.29 ^e	ND	1.09 ± 0.04 ^e	64.46 ± 1.61 ^b	4.64 ± 1.62 ^d	137.82 ± 6.57 ^b
Uncooked pasta						
CO	12.28 ± 0.20 ^a	0.24 ± 0.02 ^c	0.76 ± 0.1 ^a	32.23 ± 0.16 ^{ab}	54.48 ± 0.24 ^a	269.22 ± 0.55 ^a
CFP5	16.67 ± 0.25 ^b	0.36 ± 0.01 ^b	1.04 ± 0.07 ^b	32.74 ± 0.11 ^{ab}	49.08 ± 0.31 ^b	266.72 ± 0.52 ^{ab}
CFP10	20.08 ± 0.26 ^c	0.39 ± 0.02 ^b	1.28 ± 0.06 ^c	33.02 ± 0.16 ^a	45.13 ± 0.35 ^c	264.76 ± 0.88 ^{ab}
CFP15	25.29 ± 0.14 ^d	0.53 ± 0.04 ^a	1.53 ± 0.00 ^d	33.24 ± 0.44 ^a	39.32 ± 0.43 ^d	263.57 ± 1.83 ^{ab}
CFP20	30.12 ± 0.06 ^e	0.55 ± 0.01 ^a	1.69 ± 0.05 ^e	31.98 ± 1.04 ^b	35.63 ± 0.99 ^e	268.04 ± 3.96 ^b

CFP5, CFP10, CFP15, and CFP20: pasta prepared with 5, 10, 15, and 20 g of fish powder /100 g of semolina flour. CO: control sample.

Results in the table represent the mean of triplicate measurements.

Mean ± standard deviation. Values within a group column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test. ND: not detected

The fish powder incorporation decreased ($P < 0.05$) the carbohydrates and moisture content whereas increased ($P < 0.05$) the lipid, protein, and ash; potentially due to the fish powder composition. The decrease in the moisture content can be attributed to a greater protein-polysaccharides interaction when compared to control (Gomez-Guillen, Borderias, & Montero, 1997; Zhang, Li, Wang, Xue, & Xue, 2016). Previous research has also shown an increase in protein, ash and lipid contents when *Catla catla* mince, *Sardinella longiceps* mince and oil, tilapia protein concentrate and tilapia flour were

added to pasta formulations, respectively (Anbudhasan & Surendraraj, 2014; Goes et al., 2016; Lakshmi Devi et al., 2013; Monteiro et al., 2016). Pasta incorporation with 20 g/100g of fish powder showed the greatest ($P < 0.05$) energy value, however no difference ($P > 0.05$) was observed between CFP 5 g/100g, CFP10 g/100g and CFP 15 g/100g in the energy value. The lowest energy ($P < 0.05$) value was observed in the control sample as compared to the enriched pasta with fish powder. This increase in energy value in pasta enriched with fish powder could be due to the increase of protein. The enrichment in cod is known to allow the inclusion of nutrients such as polyunsaturated fatty acids and essential amino acids (Oliveira et al., 2015)) but absent from the durum wheat semolina (Zhang et al., 2016).

4.3.2 Effect of fish powder inclusion on cooking loss, swelling index and water absorption index of pasta

The cooking quality of pasta is an important feature and is assessed using optimal cooking time, cooking loss (solid material leaching during cooking), water absorption index, and swelling index which represents the uptake of water content during cooking. Several authors have reported that the quality and content of protein used in pasta processing as well as protein interaction in the continuous network, are very important to form the optimum carbohydrates - protein network in order to obtain pasta of good cooking quality the best being gluten-forming proteins (Chillo et al., 2010; Cleary & Brennan, 2006; Noni & Pagani, 2010). Table 4.2 shows that increasing levels of fish powder in pasta resulted in increased cooking loss compared with the control pasta.

Table 4.2 Physical properties of cooked pasta products enriched with fish powder.

Sample	Optimal Cooking Time (min)	Cooking Loss (g/100g)	Swelling Index (g water/ g dry pasta)	Water absorption Index (g/100g)
CO	6.30	3.99 ± 0.27 ^{bc}	2.95 ± 0.09 ^a	98.91 ± 6.20 ^a
CFP5	5.30	3.54 ± 0.12 ^c	1.91 ± 0.09 ^b	87.84 ± 4.15 ^{ab}
CFP10	5.30	3.97 ± 0.11 ^{bc}	1.93 ± 0.05 ^b	85.42 ± 3.21 ^b
CFP15	5.00	4.55 ± 0.42 ^b	1.92 ± 0.06 ^b	83.97 ± 2.99 ^b
CFP20	5.00	5.85 ± 0.40 ^a	1.81 ± 0.14 ^b	74.11 ± 6.15 ^c

CFP5, CFP10, CFP15, and CFP20: pasta prepared with 5, 10, 15, and 20 g of fish powder /100 g of semolina flour. CO: control sample.

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The highest cooking loss values were for the 20 g/100g enriched pasta samples (5.85 g/100 g), while the sample with no fish powder had significantly ($P < 0.05$) lower cooking loss (3.99 g/100 g). However, all pasta samples presented cooking losses below 8 g/100 g, the value above which pasta quality is considered unacceptable according to industry guidelines (Foschia et al., 2015a ; Dick & Young, 1988). The higher cooking loss in pasta enriched with fish powder might be attributed to a weakening and disruption of the protein gluten network. Similar results have been observed by Ramya et al. (2015) who studied the effect of shrimp meat powder on the leaching of solids from pasta and reported that the solids that leached into the cooking water increased as the inclusion level of shrimp meat powder was increased. Also, Chin et al. (2012) who worked on the effect of inclusion of threadfin bream (*Nemipterus sp.*) powder on leaching of solids, found that as the inclusion levels increased in pasta (5-20 g/100g), the cooking loss increased from 13.51-19.45 %. These results were in agreement with those from Vijaykrishnaraj et al. (2014) and (Kadam & Prabhasankar, 2012) who reported an

increase in cooking loss of pasta containing 2.5-10 g/100g green mussel powder and 10-30 g/100g shrimp meat.

The optimum cooking time decreased with the addition of fish powder to pasta samples (Table 4.2). The reduction in cooking time was due to a lower water absorption (98.91 % for control and 87.84 % to 74.11 % for pasta enriched fish powder) and higher cooking loss (3.99 % for control vs 3.54 % to 5.85 % for enriched pasta). These results are in agreement with (Petitot et al., 2010) and (de la Peña & Manthey, 2014) who also reported that pasta fortified with bean flour and soybean flour had a shorter cooking time than control pasta. In contrast to this study, other authors have reported that pasta containing shrimp meat had minimal or longer cooking time than the control pasta (Kadam & Prabhasankar, 2012; Lakshmi Devi et al., 2013). The swelling index of pasta samples are reported in Table 4.2. Pasta prepared with 5–20 g/100g fish powder showed significantly lower swelling index (1.91-1.81 g water/g dry pasta respectively) than the control pasta (2.95 g water/g dry pasta). The reduced swelling index could be due to the formation of a protein network in the pasta enriched with fish powder resulting in the limited supply of water for starch granule for swelling and gelatinisation. Similar results were observed by Liu et al. (2016) who reported that swelling index decreased significantly ($P < 0.05$) as the levels of meat (15-45 g/100 g) increased in fortified pasta. However, some research has shown a significant increase in the swelling index at increasing concentration of dietary fibre and legumes in pasta (Aravind, Sissons, Fellows, Blazek, & Gilbert, 2012b; Brennan & Tudorica, 2007; Brennan et al., 2004; Cleary & Brennan, 2006; Foschia et al., 2015a; Wojtowicz & Moscicki, 2014). The difference in optimal cooking time and swelling index results obtained in the present study and reported in literature could be due to different type and content of

ingredients and different processes used (Brennan et al., 2013b; Noni & Pagani, 2010). Water absorption index is a measure of the amount of water absorbed by the pasta (Oikonomou & Krokida, 2011). Table 4.2 illustrates that the substitution of semolina flour with fish powder caused a significant decrease in water absorption index. Water absorption value ranged from 87.84 g/100g to 74.11 g/100 g for pasta containing 5-20 g/100g fish powder respectively and was 98.91 g/100g for control. This may be due to the substitution of semolina flour with fish powder in pasta samples, which reduces starch swelling and pasta water absorption by competing with the starch for water during pasta formation. The decrease in water absorption index could be partly explained by a decrease in swelling index. During pasta formation, fish powder is competing with the starch for water and this would reduce starch swelling and consequently water absorption of pasta. Similarly, (Ramya et al. 2015) and (Vijaykrishnaraj et al., 2014) reported that the addition of shrimp meat and green mussel powder in pasta significantly ($P < 0.05$) decreased water absorption index as the level of shrimp meat and green mussel powder increased in the blend. More recently, (Vijaykrishnaraj et al., 2014) found that pasta water absorption was affected by inclusion with 2.5 -10 g/100g of green mussel powder. In contrast to this study, (Devi et al., 2013) reported an increase in water absorption value of pasta containing 15 g/100g fish mince. The higher water absorption index values obtained for pasta containing fish mince may be explained by the higher capacity of the fish mince to absorb and retain water within a very well developed starch-protein network. Recently, (Foschia et al., 2015a) reported that inclusion of different dietary fibre into pasta cause a significant increase in water absorption index than control semolina sample. Brennan et al. (2004) reported that the water absorption index of pasta increased due to the

increased degree of starch gelatinisation and disruption of the protein-starch matrix within the product. In this study, the results of the water absorption indicated that starch in the pasta enriched with fish powder may be less gelatinised during pasta cooking compare to the control sample.

4.3.3 Colour measurement of pasta

The colour parameter of pasta is an important factor responsible for consumer acceptance (Petitot et al., 2010). Table 4.3 and Figure 4.1 shows the L^* , a^* and b^* values for all pasta samples before and after cooking. Uncooked and cooked pasta samples enriched with fish powder showed lower lightness (L^*) value than control pasta.

Table 4.3 Colour characteristics of cooked and uncooked pasta enriched with fish powder.

Sample	Measurements			
	L^*	a^*	b^*	ΔE
Uncooked pasta				
CO	90.24 ± 0.76 ^a	-8.08 ± 0.24 ^a	29.35 ± 0.35 ^b	
CFP5	89.97 ± 0.92 ^a	-9.48 ± 0.72 ^b	31.02 ± 0.61 ^{ab}	2.55 ± 0.66 ^a
CFP10	89.88 ± 0.97 ^a	-9.17 ± 0.20 ^b	31.24 ± 0.30 ^a	2.50 ± 0.47 ^a
CFP15	90.75 ± 0.51 ^a	-9.50 ± 0.05 ^b	31.31 ± 0.02 ^a	2.66 ± 0.48 ^a
CFP20	89.94 ± 1.76 ^a	-9.54 ± 0.15 ^b	30.26 ± 1.21 ^{ab}	2.93 ± 0.69 ^a
Cooked pasta				
CO	91.24 ± 0.23 ^a	-9.59 ± 0.09 ^a	26.81 ± 0.77 ^b	
CFP5	90.52 ± 0.61 ^{ab}	-10.79 ± 0.56 ^a	28.81 ± 0.28 ^a	2.49 ± 1.01 ^a
CFP10	89.49 ± 1.52 ^{abc}	-10.26 ± 0.42 ^a	29.01 ± 0.87 ^a	2.99 ± 1.63 ^a
CFP15	87.84 ± 0.51 ^c	-10.06 ± 0.15 ^a	28.08 ± 0.47 ^{ab}	3.82 ± 0.40 ^a
CFP20	88.63 ± 1.15 ^{bc}	-10.13 ± 0.85 ^a	27.86 ± 0.68 ^{ab}	3.29 ± 0.63 ^a

CFP5, CFP10, CFP15, and CFP20: pasta prepared with 5, 10, 15, and 20 g of fish powder /100 g of semolina flour. CO: control sample.

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The lightness of pasta samples decreased as the amount of fish powder in the recipe increased. This observation was more evident for cooked pasta with addition of 15 and

20 g/100g fish powder ($P < 0.05$). Similarly, Kadam & Prabhasankar (2012) studied the effect of shrimp meat on the colour characteristics of pasta and reported that the addition of 10-30 g/100g shrimp meat into pasta decreased the lightness (L^*) value compared to control samples.

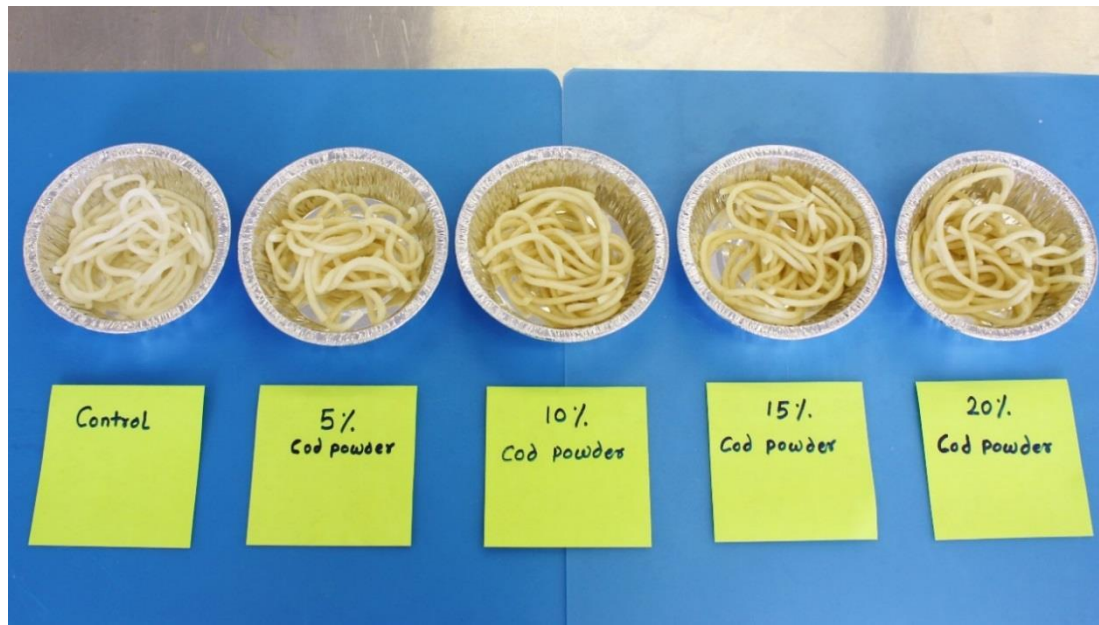


Figure 4.1 Pasta enriched with different levels of cod powder.

In addition, Vijaykrishnaraj et al. (2014) and (Liu et al., 2016) found that increased levels of green mussel powder and meat in pasta showed decreased lightness parameters. The increase redness parameter (a^*) in pasta enriched with fish powder showed a significant increase ($P < 0.05$) compared to control samples while in cooked pasta samples redness (a^*) is not affected by the treatments. These results were in agreement with those from Kadam & Prabhasankar (2012) and Vijaykrishnaraj et al. (2014), who reported an increase in the red colour of pasta associated with the inclusion level of shrimp meat and green mussel powder. The yellowness b value was compared to understand the acceptability of product; the b^* values for uncooked and cooked pasta samples were 29 to 31 and 26 to 29, respectively. Changes in colour among different

pasta samples were due to various incorporation levels. The results obtained by Ramya et al. (2015) supports the above mentioned observation of low L^* and b^* values. They reported that yellowness value of pasta samples increased as the level of shrimp meat (2.5 to 10 g/100g) powder increased in pasta. Also, (Santana et al., 2015) demonstrated that incorporation of 50 and 100 g/100g surimi powder in sausages were significantly different yellowness (b^*) characteristics to those of control. This difference may be due to the higher concentration of surimi powder used.

The ΔE values was also determined to evaluate the colour differences between the control and the fish powder containing formulations. The ΔE values of fish powder containing pasta increased with increasing levels of fish powder in both cooked and uncooked forms. In addition, cooked pasta exhibited higher ΔE compared to the uncooked pasta, indicative of the colour compounds released after cooking of pasta. The ΔE values were more than 3.0 for cooked pasta, and below 3.0 for uncooked pasta. According to handbook of colour science, these values fall in the “appreciable, detectable by ordinary people” and “noticeable, detectable by trained people” respectively. Khan et al.(2014) reported that sorghum flour enriched pasta showed higher ΔE with increasing level 20% to 40%.

4.3.4 Textural measurements

For pasta cooking, quality texture parameters are important characteristics. From the consumer point of view, the development of texture parameters is a critical point to ensure the acceptance of products. The textural properties of pasta are mainly controlled by a gluten network, which is a structural network of starches, protein additions, and other ingredients (Chang & Wu, 2008).



Figure 4.2 Texture analyser and probe used for pasta firmness and extensibility.

Firmness is a reflection of the bond strength and the integrity of the protein matrix present in the pasta after the cooking process (Phongthai, D'Amico, Schoenlechner, Homthawornchoo, & Rawdkuen, 2017). The firmness and tension properties of the pasta enriched with fish powder and control sample are shown in Table 4.4 and Figure 4.2. There was a significant increase in firmness when the amount of fish powder was increased ($P < 0.05$). The firmness increased from 2.79 N in control sample to 3.81- 4.51 N in the 5-20 % fish powder sample respectively. The above results appeared to be related to values obtained for cooking losses, indicating that high cooking loss in 20 g/100g fish powder (5.85 %) had the highest firmness value (4.51 N). This could be due to the incorporation of fish powder in pasta, with low swelling index and water absorption index value being related to hardness.

Table 4.4 Textural properties of enriched pasta with fish powder.

Sample	Measurements	
	Firmness Peak force (N)	Maximum breaking strength (N)
CO	2.79 ± 0.05 ^c	0.42 ± 0.01 ^c
CFP5	3.81 ± 0.02 ^b	0.46 ± 0.01 ^b
CFP10	4.45 ± 0.05 ^a	0.54 ± 0.01 ^a
CFP15	4.39 ± 0.05 ^a	0.53 ± 0.01 ^a
CFP20	4.51 ± 0.13 ^a	0.56 ± 0.02 ^a

CFP5, CFP10, CFP15, and CFP20: pasta prepared with 5, 10, 15, and 20 g of fish powder /100 g of semolina flour. CO: control sample.

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

In the present study, fish protein interacted with the insoluble network of pasta, forming a matrix structure, and leading to the high firmness and extensibility as observed from results. The textural properties of pasta in the present study corresponds to results from studies carried out on the addition of shrimp meat powder and green mussel powder into pasta (Ramya et al., 2015; Vijaykrishnaraj et al., 2014), and the incorporation of fish meat, shrimp meat and beef meat into pasta (Kadam & Prabhasankar, 2012; Lakshmi Devi et al., 2013; Liu et al., 2016). (Foschia et al., 2015a) reported that higher moisture content and swelling index are responsible for lower firmness value of pasta like products. Extensibility was examined as maximum force applied before breaking pasta (Chang & Wu, 2008). The extensibility of pasta increased significantly ($P < 0.05$) as the levels of fish powder increased (Table 4.4 and Figure 4.2). This may be attributed to the higher amount of polypeptide chain associated with higher protein content, which increases the ability of proteins to form an insoluble network. This insoluble protein network can entrap swollen and gelatinised starch

granules, which prevents pasta from disruption (Chillo et al., 2010). The extensibility value in the present study ranged from 0.42 N to 0.56 N.

4.3.5 Conclusions

The results illustrated that the fish powder can be incorporated into pasta to enhance the product with high protein and other bioactive ingredients. The addition of fish powder affected cooking, textural and colour parameters. The fortification of pasta with fish powder improved the protein, fat and ash contents. The cooking loss increased and cooking time decreased with the addition of fish powder to pasta. In addition, increased firmness and extensibility were observed in a higher fish powder containing pasta. Obtained results showed that cod powder could be a beneficial additive for semolina pasta production with enhanced physicochemical properties which will help in reducing all health problems of human beings. Further deepening would be needed about the ascertainment of sensory features (odour, taste) of reformulated products and shelf life of the end product.

Chapter 5

Effect of fortification with cod fish (*Pseudophycis bachus*) powder on nutritional quality of durum wheat pasta

This chapter is published as:

Ajay S. Desai., Margaret A. Brennan., & Charles S. Brennan (2018). Effect of fortification with fish (*Pseudophycis bachus*) powder on nutritional quality of durum wheat pasta. **Foods, 7, 62-74.**

Abstract

This paper investigates the nutraceutical (phenolic content and antioxidant activity) and nutritional potential (protein and starch digestibility) of supplementation of durum wheat semolina pasta with 5-20% of fish powder (*Psydoophysis bachus*). In general, all enriched pasta with fish powder showed a significant decrease ($P < 0.05$) in reducing sugar released during an *in vitro* digestion and standardised AUC values compared to control pasta. The potentially bio-accessible fraction of pasta enriched with 20% fish powder (CFP) was characterised by a 177-191% increase in phenolic content and a 145-556% higher antiradical activity. Elevation of these parameters in fortified pasta was accompanied by interaction of wheat starch, protein and fish powder protein. Supplementation of fish powder also influenced protein digestibility (a reduction from 84.60% for control pasta to 80.80% for pasta with 20% fish powder). Fortification improved the nutraceutical and nutritional potential of the studied pasta with the effects depending on factors, including protein-starch-phenolic interactions.

5.1 Introduction

There is an increasing trend for consumers to demand the development of nutritionally rich foods while also moderating the quantity of digestible starch due to health concerns related to their high intake. A high intake of readily digestible starch results in increased blood glucose levels and may be related to obesity and being overweight (Sopade, 2017). The worldwide prevalence of diabetes is predicted to increase from 382 million people to 592 million by the end of 2035 and approximately 10% of the population will have diabetes (Guariguata et al., 2014). The search for health enhancing food ingredients for pasta preparation has been growing. A promising approach of examining the enrichment of food ingredient and their physiological effects is by the concept of glycaemic index (GI) which is used to predict postprandial blood glucose level (Brennan, Derbyshire, Tiwari, & Brennan, 2012). Consumption of foods with a low glycaemic value could manipulate the effects of diabetes cardiovascular and neurodegenerative disease (Augustin et al., 2015). Low glycaemic index (GI) foods can be achieved with the utilisation of protein rich and fibre rich ingredient combined with cereal grains in products such as bread and pasta (Brennan et al., 2013b; Morales et al., 2015). Pasta is a staple food, containing carbohydrates (74–77%) and protein (11–15%), although pasta is deficient in lysine and methionine. Pasta may be fortified with functional ingredients to alter the nutritional quality of pasta such as essential amino acids, minerals, vitamin and phenolic compounds (Brennan et al., 2013b). To achieve this, pasta products have been fortified with high protein sources, such as soya flours, soy isolates, milk and milk products, whey proteins, yeast protein concentrates and meat (Dziki et al., 2014; Torres, Frias, Granito, & Vidal-Valverde, 2006). Fortification of food is most convenient method which include addition of one or more functional

components for the purpose of enhancing a biological activity of newly designed food products (Lorusso et al., 2017). In recent years, pasta has been fortified using different ingredients including quinoa flour (Gimenez, Drago, Bassett, Lobo, & Sammán, 2016), lentil flour (Aryee & Boye, 2016), beef meat (Liu et al., 2016) and freeze dried shrimp powder (Ramya et al., 2015). The Food and Drug Administration (FDA) and World Health Organization (WHO) consider pasta a vehicle for the addition of nutrients to the diet, as it can be enriched with protein and various bioactive ingredients such as phenolic compounds and dietary fibre (Chillo et al., 2010). Phenolic compounds exhibit biological properties, such as, antioxidant activity. Food rich in polyphenols have the potential to protect against various diseases associated with oxidative damage, such as cardiovascular, cancer and neurological disease (Seczyk, Swieca, et al., 2016).

Fish is not only an excellent source of high value protein but also an important source of essential amino acids and ω -3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as containing micronutrients such as vitamins (A, D, B6 and B12) and minerals (iron, zinc, iodine, selenium, potassium and sodium). The American heart association (AHA) recommends a minimum consumption of two fish serving per week (equivalent to 200mg/day of long chain ω -3 polyunsaturated fatty acid (PUFA)) to achieve a cardio protective effect. To fulfil these requirements pasta could be fortified with fish powder. The fish powder is a protein rich which contributes to a low glycaemic index and potential to have beneficial health effects such as manipulation of obesity, hypertension and cardiovascular disease (Kadam & Prabhasankar, 2010). Previous studies have reported the nutritional and physicochemical characteristics of pasta manufactured with fish powder of green mussel (*Perna canaliculus*) (Vijaykrishnaraj et al., 2014), shrimp meat (*Penaeus*

monodon) (Ramya et al., 2015) and beef meat (Liu et al., 2016). However, the nutritional properties of pasta enriched with partial replacement of semolina wheat flour by red cod powder (*Pseudophycis bachus*) is still unknown. The present study was conducted to evaluate the effects of addition of different level of fish protein powder on the pasta characteristics including *in vitro* starch and protein digestibility and antioxidant activity.

5.2 Materials and Methods

5.2.1 Raw materials

Described in section 3.1

5.2.2 Fish powder preparation

Described in section 3.1.1

5.2.3 Pasta preparation

Described in section 3.2.2

5.2.4 Amino acid profile of semolina and fish powder

Described in section 3.4.7

5.2.5 Gastric digestibility determination using *in vitro* starch digestion process

Described in section 3.4.6

5.2.6 *In vitro* protein digestibility

Described in section 3.4.5

5.2.7 *In vitro* gastric intestinal digestion

Described in section 3.4.6

5.2.8 Total phenolic content in pasta

Described in section 3.4.12.1

5.2.9 Antioxidant activity of pasta (Oxygen Radical Absorbance Capacity

(ORAC) Assay

Described in section 3.4.12.2

5.2.10 Statistical analysis

Described in section 3.4.22

5.3 Results and discussion

5.3.1 Amino acid profile of semolina and fish powder

The amino acid profile of wheat semolina and fish powder samples are presented in Table 5.1 as compared to the bovine serum albumin protein (BSA).

Table 5.1 Amino acid profile of semolina, cod fish powder and bovine serum albumin^a.

Amino acid	S	CFP	BSA	EAA ^b
<i>Essential Amino Acids (EAA)</i>				
Methionine	4.57 ± 3.82	53.32 ± 3.27	15.96 ± 1.43	16
Phenylalanine	43.96 ± 3.36	65.43 ± 3.30	105.38 ± 6.60	38
Isoleucine	28.81 ± 2.71	78.81 ± 3.69	40.36 ± 2.96	30
Lysine	19.88 ± 1.49	202.83 ± 7.23	276.28 ± 7.57	45
Leucine	69.76 ± 7.22	185.35 ± 8.77	263.55 ± 6.52	59
Histidine	21.95 ± 1.68	40.06 ± 2.58	79.70 ± 5.50	15
Threonine	26.65 ± 2.12	84.93 ± 3.05	106.72 ± 6.79	23
Valine	35.55 ± 2.79	76.67 ± 3.34	96.76 ± 6.17	39
<i>Non-essential amino acids</i>				
Aspartic acid	23.30 ± 6.03	221.53 ± 6.80	230.83 ± 10.73	
Glutamic Acid	433.18 ± 10.36	364.63 ± 12.32	389.33 ± 16.58	
Cysteine	10.73 ± 3.95	15.92 ± 3.15	130.21 ± 6.56	
Serine	52.06 ± 5.58	99.85 ± 5.18	88.43 ± 6.44	
Glutamine	-	1.24 ± 0.05	1.15 ± 0.01	
Glycine	36.12 ± 2.97	76.89 ± 3.14	28.97 ± 1.69	
Arginine	31.40 ± 2.68	116.97 ± 5.13	115.53 ± 7.91	
Alanine	22.64 ± 3.18	129.43 ± 5.71	134.32 ± 9.19	
Taurine	-	3.23 ± 0.17	-	
Proline	90.22 ± 5.74	48.24 ± 9.10	68.15 ± 5.97	
Tyrosine	17.76 ± 1.62	59.78 ± 3.48	89.65 ± 6.00	
TEAA/TAA (%)	25.56 ± 0.50	40.89 ± 0.13	43.56 ± 0.08	

^aData are expressed as mg of amino acid per g of protein. Tryptophan was not determined. S: Semolina, CFP: cod fish powder, BSA: bovine serum albumin, TEAA/TAA: Total essential amino acids/Total amino acids, EAA: Essential amino acid. ^b Suggested profile of essential amino acid requirements for adult humans by FAO/WHO/UNU (2007).

The amino acids of fish powder was higher than semolina. The ratio of the total essential amino acids to the total amino acids (TEAA/TAA) was higher in fish powder than in the semolina. The essential amino acid content of the semolina and fish powder was compared with the recommendations made by (Millward, 2012) for adult humans. Fish powder sample exceeded the essential amino acid requirements for adult human and infants while the essential amino acid of semolina was below the adult and infant requirements. (FAO/WHO/UNU, 2007) reported that herring fish powder exceeded the essential amino acid requirements for adult humans.

5.3.2 *In Vitro* Predictive Glycaemic Response

An *in vitro* enzymatic digestion was performed to evaluate the nutritional quality of the pasta enriched with fish powder, in terms of their starch digestibility and predictive glycaemic response. The addition of fish powder into pasta decreased ($P < 0.05$) the extent of *in vitro* starch digestion compared to the control pasta (Figure 5.1).

Table 5.2 *In vitro* starch digestibility profile of control and pasta containing fish powder.

Samples	RDS (mg/g sample)	Area RDS (mg/g sample)	Total AUC (mg/g sample)
CP	208.26 ± 1.77 ^a	56.58 ± 0.23 ^a	227.87 ± 13.13 ^a
CFP5	164.15 ± 21.92 ^{ab}	45.0 ± 1.72 ^{ab}	204.23 ± 6.78 ^b
CFP10	179.39 ± 26.14 ^{ab}	48.75 ± 2.52 ^{ab}	211.32 ± 7.64 ^{ab}
CFP15	173.84 ± 9.31 ^{ab}	49.30 ± 1.15 ^{ab}	207.86 ± 0.83 ^{ab}
CFP20	152.83 ± 14.61 ^b	43.35 ± 1.14 ^b	193.99 ± 7.47 ^b

Mean ± SD ($n=3$). Values within a column followed by different small letters are significantly different ($P < 0.05$). RDS- rapidly digestible Starch, AUC- area under curve
CP-control pasta, P5-P20- pasta fortified with 5-20 % of cod fish powder, respectively.

The pasta enriched with 20% fish powder exhibited significantly the lower values ($P < 0.05$) of reducing sugar followed by 5, 15 and 10 pasta samples, while the control pasta showed the higher values at each time point during the digestion. The amount of rapidly

digestible starch (RDS) in pasta enriched with fish powder was lower than the control (Table 5.2). Chillo et al. (2010) reported that addition of protein rich soya bean flour into spaghetti significantly lowered the RDS fraction when compared to semolina spaghetti. Also, Brennan et al. (2012a) found a similar result in mushroom enriched extruded product, which showed that mushroom incorporation restricted the RDS from the fibre enriched products. Table 5.2 shows the RDS value and area under the curve in control and pasta enriched with fish powder. Thus, the *in vitro* digestion of pasta fortified with fish powder demonstrated that the rate and extent of reducing sugar release decreased. This may be due to the incorporation of protein rich ingredients into pasta could modifying the integrity of the protein network. Several researchers have studied the effect of addition protein rich ingredient into pasta on starch digestibility (Ramya et al., 2015; Sathivel et al., 2004). The protein content of enriched pasta with fish powder increased directly proportional to the increasing levels of fish powder added due to the original content of fish powder (88.54%).

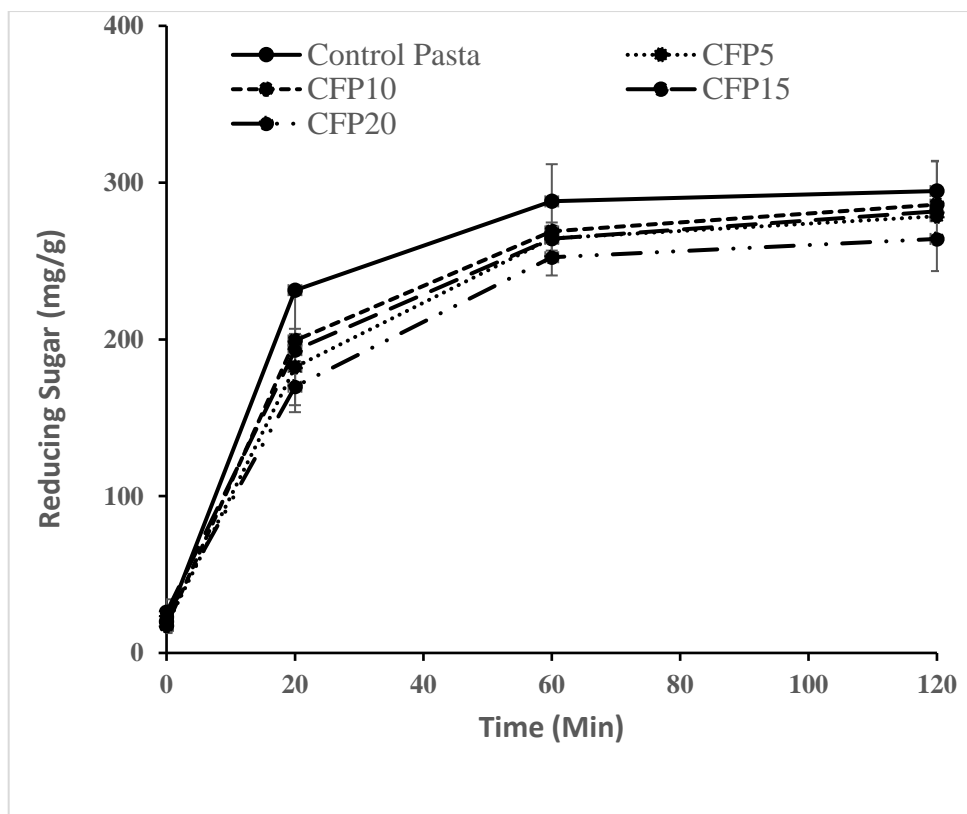


Figure 5.1 Amount of reducing sugar released during *in vitro* digestion. CP-control pasta, CFP5-CFP20 pasta fortified with 5-20% fish powder, respectively.

The addition of fish powder may create a protein network around the starch molecules and reduce the starch granules surface accessibility of α -amylase to starch and hence affect the enzyme susceptibility to hydrolysing the starch into reducing sugar. Similar results were reported by Dziki et al. (2014) who found that inclusion of 15%, 30% and 45 % beef meat into pasta exhibited a significantly decrease in reducing sugar release. Also, Ramya et al. (2015) who studied the *in vitro* starch hydrolysis of pasta made with semolina fortified with different levels (2.5%, 5% and 10%) of shrimp (*Penaeus monodon*) meat and showed a significant reduction in the reducing sugar release as the concentration of shrimp meat increased. Hager, Czerny, Bez, Zannini, & Arendt, (2013) who studied effect of the addition of oat flour to the egg pasta formulation. Pasta enriched oat flour exhibited significantly lower reducing sugar and predicated glycaemic

index at different time points compared to control. This may be due the higher addition level of egg white powder. It was reported previously that the presence of protein in food matrix creates a stronger network and reduces the capacity of enzyme attack to the starch granules, thereby delaying starch digestion (Chillo et al., 2010). Rodríguez De Marco et al. (2014) observed a similar effect on starch digestibility with addition of spirulina biomass in wheat bread pasta. They observed that spirulina formed a protein matrix as a physical barrier around the starch granules and protected them from enzyme attack. The protein rich fish powder used in this study may also decreased the reducing sugar release due to the formation of protein network which entraps the α -amylase. Table 5.2 illustrates the effects of substituting semolina flour with fish powder on standardised AUC values compared to the control pasta sample. The AUC values were decreased in pasta fortified with fish powder as compared to control. These results illustrate, again, that the fish powder enriched pasta was more resistant to starch digestion compared to the control pasta without fish powder. The addition of fish powder into pasta like product is a convenient and novel choice in lowering the glycaemic index of the final product.

5.3.3 Protein Content and *In Vitro* Protein Digestibility

Protein quality is one of the most important attributes to determine the nutritional characteristics of a food matrix and is evaluated by protein digestibility (Lorusso et al., 2017). Table 5.3 shows the values of *in vitro* protein digestibility, protein content in cooked and uncooked pasta and protein availability after digestion.

Table 5.3 Protein content, *in vitro* protein digestibility and protein availability of pasta fortified with fish powder.

Samples	PC in raw pasta (g/100g)	PC in cooked pasta (g/100g)	Significance between PC of uncooked and cooked pasta	PD (%)	PA (g/100g dry pasta)
CP	12.20 ± 0.20 ^a	12.63 ± 0.17 ^A	*	84.60 ± 0.27 ^a	10.68 ± 0.12 ^a
CFP5	16.67 ± 0.25 ^b	16.52 ± 0.29 ^B	*	82.49 ± 0.65 ^b	13.63 ± 0.31 ^b
CFP10	20.08 ± 0.26 ^c	20.69 ± 0.11 ^C	*	81.40 ± 0.54 ^{bc}	16.84 ± 0.19 ^c
CFP15	25.29 ± 0.14 ^d	25.15 ± 0.25 ^D	*	81.52 ± 0.27 ^{bc}	20.50 ± 0.12 ^d
CFP20	30.12 ± 0.06 ^e	29.82 ± 0.29 ^E	*	80.80 ± 0.37 ^c	24.09 ± 0.15 ^e

Mean ± SD (n=3). Values within a column followed by different small letters are significantly different and * indicate not significant ($P < 0.05$).

PC- protein content, PD- *in vitro* protein digestibility, PA-protein availability.

CP-control pasta, P5-P20- pasta fortified with 5-20 % of fish powder, respectively.

The authors were unable to find previous information about the *in vitro* digestibility of pasta enriched with red cod fish powder. The addition of fish powder resulted in significant ($P < 0.05$) increase protein content of pasta samples however, no significance difference ($P > 0.05$) were observed between uncooked and cooked pasta, indicating that during cooking process protein did not leach out. This result in agreement with the Rodríguez De Marco et al. (2014) who found that pasta fortified with spirulina biomass increase the protein content. Fish protein is popularly considered to have high digestibility due to the lack of strong collagenous fibre and tendons which facilitates its use for human consumption (Venugopal, 1992). However, in this study, the percentage of *in vitro* protein digestibility of pasta enriched with fish powder was significantly ($P < 0.05$) reduced (84.60 to 80.80%) as a result of the increase in the level of the fish powder. Similarly, pasta which was made with freeze dried shrimp meat replacement was shown to have a lower *in vitro* protein digestibility (72 to 90%) than a control (93

%) (Ramya et al., 2015). Rodríguez De Marco et al. (2014) also reported that *in vitro* protein digestibility of spirulina enriched pasta decreased significantly (80.88 to 55.45%) as the incorporation level increased (5 to 20%) proposing that the reduction in digestibility was due to the phenolic compound present in the spirulina. Protein digestibility in food matrix depends on factor such as polysaccharides and phenolic molecules. The interaction of phenolic compounds with protein may lead to change the digestibility. It has been proposed that oxidized phenolic compounds may react with proteins and form insoluble complexes, inhibiting the activity of proteolytic enzymes and interfering with utilisation of proteins (Labuckas, Maestri, Perelló, Martínez, & Lamarque, 2008). The pH drop curves obtained from enriched pasta with fish powder by using three enzyme (trypsin, α -chymotrypsin and protease) system are shown in Figure 5.2.

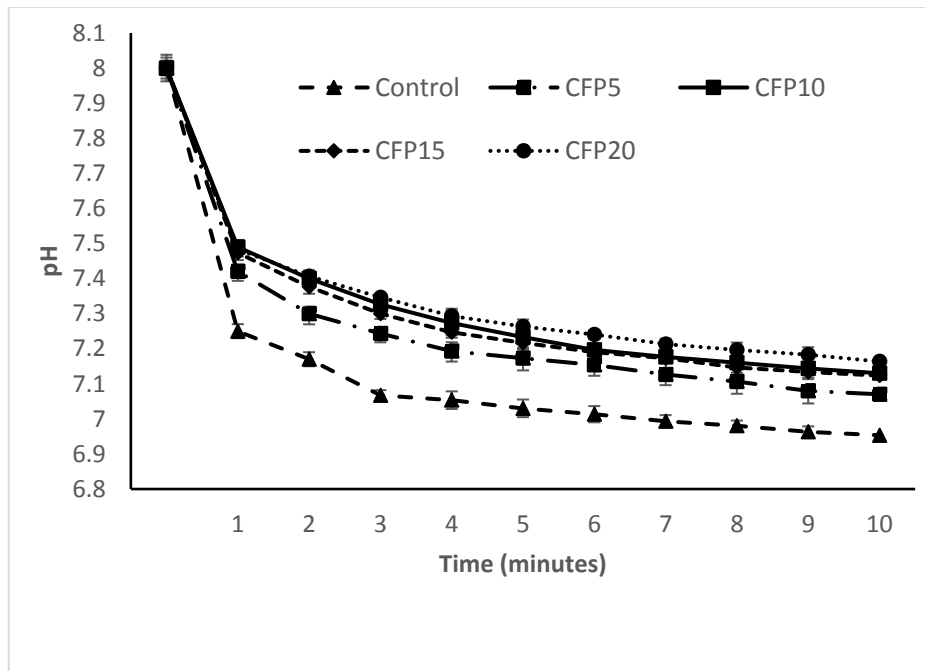


Figure 5.2 The pH vs time curves obtained by pasta made with different concentration of fish powder incubated with multi-enzymes (trypsin, chymotrypsin and protease).

The drop in pH results from the release of amino acids and peptides, protein building units, as protein are digested. After addition of multi-enzymes in protein solution, carboxyl ($-\text{COO}^-$) and amino ($-\text{NH}_3^+$) groups released. At neutral pH (8.0), the free amino groups deionize in water and protons (H^+) are liberated. The free H^+ released into the surrounding reaction medium cause as decrease in pH. At alkaline pH, phenolic compound present in enriched pasta could be oxidised by oxygen with side amino groups of peptides to form quinones and this lead to formation of protein cross-links. These quinones react with sulfhydryl and amino groups of proteins, result into decreased protein digestibility (Prodpran et al., 2012).

5.4 Phenolic content and antioxidant activity

From the consumer's point of view, after product palatability the most important factor is bioaccessibility of food components. In the present study, the *in vitro* digestion process showed fortified pasta with fish powder release significant amount of antioxidant. Fish protein have bioactive properties, but they are not so extensively studied as the peptides from other sources such as milk (Rustad, Storro, & Slizyte, 2011). In general, consumption of fish has several health beneficial effects because of the high content of easily digestible bioactive peptides (Kim & Mendis, 2006). The results describing the effect of pasta fortification on its phenolic content and antioxidant activity are presented in Table 5.4.

Table 5.4 Total phenolic content and antioxidant activity of fortified pasta subjected to *in vitro* digestion.

Sample	TPC (mg GAE/g sample)		ORAC (μmol TE/g sample)	
	(Gastric digestion)	(Pancreatic digestion)	(Gastric digestion)	(Pancreatic digestion)
	(0-30 min)	(0-120 min)	(0-30 min)	(0-120 min)
CP	1.56 ± 0.12 ^c	2.73 ± 0.08 ^a	4.39 ± 0.42 ^d	68.97 ± 4.00 ^d
CFP5	1.92 ± 0.02 ^{bc}	3.34 ± 0.04 ^b	11.07 ± 1.75 ^c	73.38 ± 8.63 ^{cd}
CFP10	2.04 ± 0.12 ^b	3.95 ± 0.16 ^c	12.84 ± 0.86 ^c	82.65 ± 3.83 ^{bc}
CFP15	2.74 ± 0.15 ^a	4.47 ± 0.09 ^d	20.09 ± 0.55 ^b	91.83 ± 3.98 ^{ab}
CFP20	2.77 ± 0.25 ^a	5.23 ± 0.36 ^e	24.45 ± 1.41 ^a	99.31 ± 1.97 ^a

Mean ± SD (n=3). Values within a column followed by different small letters are significantly different ($P < 0.05$).

TPC- total phenolic content, ORAC- oxygen radical absorbance capacity, GAE- gallic acid equivalent, TE-Trolox equivalent value.

CP-control pasta, P5-P20- pasta fortified with 5-20 % of fish powder, respectively.

Phenolic content and antioxidant activity were positively associated with the percentage of fish powder addition, and the highest values were obtained for pasta fortified with 20% supplement. In comparison to the control, after gastric and pancreatic digestion, the amount of bio-accessible total phenolic compound in fortified pasta were significantly ($P < 0.05$) higher from 1.92 to 2.77mg of gallic acid/g of pasta (representing an increase of 123% to 177%) and 2.73 to 5.23 mg of gallic acid/g of pasta (as increase of 122% to 191%) respectively indicating that adding fish powder ingredients is alternative to enhance its antioxidant activity. Antioxidant activity was observed by oxygen radical absorbance capacity (ORAC) mechanism. An elevation from 4.39 to 24.45 μmol Trolox / g of pasta (gastric digestion) and 68.97 to 99.31 μmol Trolox / g of pasta (pancreatic digestion) by supplemented pasta (5-20%) was observed. The bioaccessibility of phenolic compounds after digestion varied according to the enriched pasta with fish powder. The total phenolic content and antioxidant activity of control sample was lower (1.56 mg of gallic acid/g of pasta) than the fortified pasta. This could

be due to leaching of phenolic compounds into the cooking medium with higher cooking time. However, retention of phenolic content was significantly higher in fish powder containing pasta as compared to control samples ($P < 0.05$). This clearly indicates that incorporation of fish powder results in retaining phenolic compounds in the pasta upon cooking. However, Khan et al. (2014) observed a significant decrease in total phenolic content in cooked sorghum fortified pasta compared to raw formulations, as observed by Prabhasankar et al. (2009) in seaweed enriched pasta. Both researchers were agreed that during cooking, phenolic compounds leached into the cooking medium and degraded due to thermal treatment. The loss of antioxidant activity due to cooking processes has been reported in other investigations Cardenas-Hernandez et al. (2016) which suggest that during cooking there is more leaching of bioactive compounds from pasta with durum wheat semolina (Fares, Platani, Baiano, & Menga, 2010). In present study, the pasta fortified with fish powder was able to retain phenolic compound upon cooking. Similar positive correlations between phenolic level, antioxidant activity and the supplemental level of fortified pasta have been recorded previously (Biernacka, Dziki, Gawlik-Dziki, Różyło, & Siastała, 2017; Cardenas-Hernandez et al., 2016; Rodríguez De Marco et al., 2014; Sęczyk, Świeca, Gawlik-Dziki, Luty, & Czyz, 2016; Vijaykrishnaraj et al., 2014). For instance, results obtained by Vijaykrishnaraj et al. (2014) show an increase in ability to reduce 132% DPPH radical activity of cooked pasta enriched with 5% of green mussel powder. On the other hand, Ozdal, Capanoglu, & Altay (2013) reported that protein and phenol interact with each other through covalent or non-covalent interaction. These interactions might lead to the precipitation of protein from food matrix with studies from Rawel, Meidtner, & Kroll (2005) showing that covalent bonding may affect both secondary and tertiary structure of protein.

Besides the type and level of functional ingredients used, other factors such as processing and cooking are responsible for alteration of antioxidant properties of pasta (Sun-Waterhouse, Jin, & Waterhouse, 2013). Additionally, the presence of oxygen, water and heat treatment during cooking and pasta making may induce the oxidation of sensitive phenolic antioxidants (Khan et al., 2013).

5.5 Conclusions

This study demonstrated that the addition of fish powder in pasta, is an effective method to enhance essential amino acid, protein content, the starch digestibility and the antioxidant potential. This may be beneficial to prevent the outbreaks of chronic disease related to oxidative stress such as type 2 diabetes and for improved intestinal health. The significant reduction in glucose release during *in vitro* digestion of pasta fortified with fish powder compared to the control indicates that there is potential of using protein rich fish powder in pasta making. Moreover, antioxidant activity from supplemented pasta are highly bio-accessible *in vitro*. However, the quality of fortified pasta is affected by multiple factors, protein-starch and protein-phenol interactions. These interactions between fish powder protein and durum wheat starch and protein influenced antioxidant activity, starch and protein digestibility of fortified pasta. In addition, further work is required to evaluate the consumer acceptability. In summary, to develop pasta like product with protein rich ingredients, knowledge of the interaction existing between protein-starch-phenolic in food matrix is necessary.

Chapter 6

Influence of semolina replacement with salmon (*Oncorhynchus tshawytscha*) powder on the physicochemical attributes of fresh pasta

This chapter is published as:

Ajay S. Desai., Margaret A. Brennan., & Charles S. Brennan (2018). Influence of semolina replacement with salmon (*O.tshawytscha*) powder on the physicochemical attributes of fresh pasta. **International Journal of Food Science and Technology**, 53 (6), 1425-1435.

Abstract

This study evaluated the influence of the incorporation of salmon (*Oncorhynchus tshawytscha*) fish powder (SFP) into pasta production and the effect on pasta physicochemical attributes. Four replacement levels were tested (5%, 10%, 15% and 20%) together with a control pasta (100% semolina). The effects on the chemical composition, physical properties (optimal cooking time, cooking loss, water absorption index, swelling index and colour and textural properties (firmness and extensibility) were analysed. The results demonstrated that pasta with SFP had increased protein (12.88%-23.40%), lipid (0.46%-7.20%), ash (0.39%-0.57%) and energy (122.26-161.08 kcal) contents ($P < 0.05$), increased cooking time (6.30-8.30 min) and cooking loss (4.28%-8.02%) compared with semolina control pasta. However, all pasta samples were in the acceptable range (8 g/100g) for cooking loss. The addition of SFP resulted in significantly decreased swelling index (2.29%-1.95), water absorption (105.46%-81.62%) and firmness (3.13-1.16 N) ($P < 0.05$) whilst increasing resistance to uniaxial

extension of pasta. Colour parameters indicated comparable brightness between the samples and higher redness values for enriched pasta. Thus, pasta fortified with SFP has the potential to be a technological alternative for the food industry to provide protein enriched pasta.

6.1 Introduction

Pasta is widely consumed all over the world due to its low cost, easy preparation and nutritional quality (Foschia et al., 2015b; Lu et al., 2016). High quality pastas are generally prepared using durum wheat semolina (Biernacka et al., 2017) and contain protein, vitamins and carbohydrates (Foschia et al., 2015a; Krishnan et al., 2012). The overall quality of pasta is determined by cooking properties such as optimal cooking time (OCT), cooking loss (CL), water absorption index (WAI), swelling index (SI), (Ficco et al., 2016 ; Sobota et al., 2015), and overall textural characteristics. Pasta quality and cooking characteristics are dependent on protein-starch, protein-lipid and other component interactions (El-Khayat et al., 2006) which play an important role in the formation of three dimensional network (Silva, Ascheri, Carvalho, Takeiti, & Berrios, 2014). Inclusion of lipids in pasta change its cooking quality (cooking time, swelling index, and firmness) due to the formation of amylose-lipid complex (De Pilli, Derossi, Talja, Jouppila, & Severini, 2011). Raw material composition used for the preparation of pasta product affects the physical, chemical and textural properties of pasta (Lu et al., 2016). The World Health Organization (WHO) and Food Agriculture Organisation (FAO) both consider pasta as suitable for the incorporation of different nutrients, since it can be fortified with protein, lipid, vitamins and minerals (Chillo et al., 2010). There are many studies that have focused on increasing the nutritional value of pasta in terms of

the protein content (de la Peña & Manthey, 2014; Lorusso et al., 2017; Martínez, Marín, Gili, Penci, & Ribotta, 2017). Over the past few decades, wheat pasta has been prepared incorporating different ingredients including, millet flour (Gull, Prasad, & Kumar, 2015); shrimp meat (Ramya et al., 2015), shrimp mince (Kadam & Prabhasankar, 2012), beef meat (Liu et al., 2016), green mussel (Vijaykrishnaraj et al., 2014), tomato matrix or bran extracts, (Pasqualone et al., 2016) artichoke extracts (Pasqualone et al., 2017), broad bean and quinoa (Gimenez et al., 2016) and legume flour (Bouasla et al., 2017). Previous research has demonstrated that it is possible to enrich a starch based pasta product by the addition of fish proteins and lipids (Singh et al., 2014). In 2015, the seafood export revenue was NZ\$ 1452 million, out of which NZ\$ 47 million was derived from salmon. Salmon exports from the New Zealand has been steadily increasing and the major export markets include Canada, Japan, Hong Kong, Australia and USA (Seafood New Zealand, 2015). Salmon is rich in beneficial long chain ω -3 polyunsaturated fatty acids (LC ω -3PUFAs) namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and high quality proteins. A high intake of LC ω -3PUFAs promotes beneficial effects in human health including reducing the impact of cardiovascular diseases, diabetes, cancer, obesity, asthma and depression (Cascant et al., 2018). Fish species are an important source of nutrients such as vitamins (A, D, B6 and B12) and minerals (iron, zinc, iodine, selenium, potassium and sodium (Matos et al., 2015). The WHO and FAO recommend a regular fish consumption of 1-2 servings per week in order to provide an equivalent of 200-500 mg of LC ω -3PUFAs. Researchers have reported that the amount of ω -3 fatty acids in the diet is approximately 0.15 g per day, which is far below recommended level (Bannenberg et al., 2017). EPA and DHA PUFAs cannot be synthesised by the human body and needs to be ingested as part of a healthy diet from

external source with diet (Ortiz et al., 2013). Fish powder is a product of fish processing and represents a cheap source of high quality nutrients that can be utilised in the human diet (Oliveira et al., 2015). Previous studies have evaluated the nutritional and physicochemical characteristics of pasta manufactured with fish powder derived from shrimp meat (Kadam & Prabhasankar, 2012), *Nemipterus Japonicus* (Chin et al., 2012), *Catla Catla* mince (Lakshmi Devi et al., 2013), green mussel (*Perna canaliculus*) powder, (Vijaykrishnaraj et al., 2014), *Oreochromis niloticus* (Monteiro et al., 2016). However, nutritional composition and chemical stability varies dependent on the fish species that is utilised and processing parameters employed (Schneedorferova et al., 2015). The aim of this project was to determine the effects of substituting semolina flour with salmon powder in terms of the changes in nutritional, cooking, colour and textural characteristics of the pasta.

6.2 Materials and methods

6.2.1 Raw materials

Described in section 3.1

6.2.2 Salmon fish powder (SFP) preparation

Described in section 3.1.1

6.2.3 Pasta production

Described in section 3.2.2

6.2.4 Proximate chemical composition analysis of pasta

Described in section 3.4.1 to 3.4.4

6.2.5 Physical properties

6.2.5.1 Optimal cooking time

Described in section 3.4.13.1

6.2.5.2 Cooking loss

Described in section 3.4.13.2

6.2.5.3 Swelling index (SI)

Described in section 3.4.13.3

6.2.5.4 Water absorption index (WAI)

Described in section 3.4.13.3

6.2.6 Colour measurements

Described in section 3.4.13.4

6.2.7 Textural measurements

Described in section 3.4.11.5

6.2.8 Moisture content

Described in section 3.4

6.2.9 Statistical analysis

Described in section 3.4.22

6.3 Results and discussion

6.3.1 Chemical composition of pasta enriched with SFP

The chemical composition of salmon powder enriched cooked and uncooked pasta are shown in Table 6.1. The addition of protein rich fish powder to semolina has been shown to affect the functional properties of pasta (Chillo, Laverse, Falcone, & Del Nobile, 2008). The incorporation of SFP resulted in level-dependent effects ($P < 0.05$) on the chemical composition of the pasta formulations. The carbohydrate and moisture content decreased ($P < 0.05$) whereas the levels of protein, lipid, and ash increased ($P < 0.05$) with increasing levels of SFP. A similar trend of an increase in protein, lipid, and ash contents has been reported for pasta made with shrimp meat (Kadam & Prabhasankar,

2012), green mussel (Vijaykrishnaraj et al., 2014), tilapia (*Oreochromis niloticus*) flour (Monteiro et al., 2016), broad bean (Giménez et al., 2016), quinoa flour (Lorusso et al., 2017), and legume flour (Bouasla et al., 2017).

Table 6.1 Chemical composition (g/100g) (on an as-is basis) and energy value (kcal/100g) of cooked and uncooked pasta enriched with different salmon fish powder (SFP) levels.

Sample	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Moisture (g/100g)	Carbohydrate (g/100g)	Energy (Kcal/100g)
Fish powder	58.06 ± 0.13	38.55 ± 0.09	1.37 ± 0.06	2.15 ± 0.03	-	579.55 ± 0.30
Semolina	12.7 ± 0.05	1.0 ± 0.01	1.1 ± 0.02	10 ± 0.10	72.8 ± 0.24	350.8 ± 2.56
Uncooked pasta						
CP	12.60 ± 0.05 ^a	1.15 ± 0.03 ^e	0.60 ± 0.02 ^e	33.02 ± 0.09 ^a	52.62 ± 0.16 ^a	271.26 ± 0.25 ^e
SFP5	14.34 ± 0.03 ^b	3.65 ± 0.08 ^d	0.78 ± 0.01 ^d	31.66 ± 0.23 ^b	49.57 ± 0.16 ^b	288.45 ± 0.38 ^d
SFP10	17.67 ± 0.04 ^c	5.20 ± 0.02 ^c	0.89 ± 0.01 ^c	32.07 ± 0.11 ^b	44.17 ± 0.12 ^c	294.15 ± 0.40 ^c
SFP15	20.73 ± 0.10 ^d	7.25 ± 0.05 ^b	1.05 ± 0.01 ^b	31.78 ± 0.23 ^b	39.17 ± 0.21 ^d	304.85 ± 0.90 ^b
SFP20	22.70 ± 0.30 ^e	9.16 ± 0.01 ^a	1.22 ± 0.03 ^a	31.78 ± 0.09 ^b	35.13 ± 0.43 ^e	313.80 ± 0.88 ^a
Cooked pasta						
CP	12.88 ± 0.06 ^a	0.46 ± 0.02 ^e	0.39 ± 0.01 ^c	69.62 ± 0.92 ^a	16.63 ± 1.00 ^a	122.26 ± 3.68 ^c
SFP5	15.41 ± 0.17 ^b	1.65 ± 0.15 ^d	0.48 ± 0.04 ^b	69.64 ± 0.71 ^a	13.11 ± 0.83 ^b	128.95 ± 2.70 ^c
SFP10	18.10 ± 0.11 ^c	3.28 ± 0.28 ^c	0.52 ± 0.02 ^{ab}	66.09 ± 0.92 ^b	12.00 ± 0.99 ^b	149.95 ± 4.31 ^b
SFP15	20.77 ± 0.09 ^d	5.61 ± 0.27 ^b	0.56 ± 0.01 ^a	66.69 ± 0.83 ^b	6.37 ± 0.91 ^c	159.05 ± 2.85 ^a
SFP20	23.40 ± 0.13 ^e	7.20 ± 0.17 ^a	0.57 ± 0.03 ^a	68.15 ± 0.46 ^{ab}	0.67 ± 0.39 ^d	161.08 ± 2.58 ^a

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The decrease in the moisture content may be attributed to a change in the protein-starch interaction when compared to control pasta; the interaction between the different and increased proteins and the starch may have resulted into the entrapment of water molecules through electrostatic forces, and subsequently the more homogenous network developed with less free water (Zhang et al., 2016). Pasta fortified SFP with had significant greater ($P < 0.05$) energy value in both cooked and

uncooked samples than control pasta. This increase in energy value in pasta enriched with salmon powder may be due to the increase of lipids.

6.3.2 Effect of SFP inclusion on cooking loss, swelling index and water absorption index of pasta

The cooking quality of pasta is assessed using optimal cooking time, cooking loss, water absorption index, and swelling index which represents the uptake of water content during cooking. Cleary and Brennan (2006), (Giuberti, Gallo, Cerioli, Fortunati, & Masoero, 2015), and Lorusso et al. (2017) have all reported that the quality and content of protein used in pasta processing, as well as protein interactions in the continuous network, are important in the formation of carbohydrate - protein networks in order to obtain pasta of good cooking quality.

Table 6.2 Physical properties of cooked pasta products enriched with salmon fish powder (SFP).

Samples	Optimal Cooking Time (min)	Cooking Loss (g/100g)	Swelling Index (g water/ g dry pasta)	Water absorption Index (g/100g)
CP	6.30 ± 0.02	4.28 ± 0.0 ^d	2.29 ± 0.09 ^a	105.46 ± 6.51 ^a
SFP 5	8.30 ± 0.05	5.37 ± 0.61 ^c	2.26 ± 0.06 ^a	103.47 ± 2.72 ^a
SFP 10	8.00 ± 0.04	5.43 ± 0.18 ^c	1.95 ± 0.07 ^b	81.62 ± 6.31 ^b
SFP 15	7.30 ± 0.02	6.68 ± 0.20 ^b	2.00 ± 0.07 ^b	83.98 ± 4.12 ^b
SFP 20	7.00 ± 0.01	8.02 ± 0.49 ^a	2.14 ± 0.04 ^{ab}	85.71 ± 2.74 ^b

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$) according to Tukey's test.

The cooking loss of the control pasta was found to be 4.28 g/100g. Cooking losses increased ($P < 0.05$) with increasing levels of SFP ranging from 5.37 to 8.02 g/100g. However, all pasta samples presented cooking losses below the technological

acceptable limit 8 g/100 g. For good quality pasta, the residue should not exceed 8 % of the dry weight of the pasta (Smatanová & Lacko-Bartošová, 2014) and values obtained were within the limits. Semolina proteins mainly composed of glutenins and gliadins which are responsible of intra and inter-molecular disulphide bound during processing of pasta. On the other hand, salmon fish proteins are essentially composed of actin and myosin proteins (Ryan et al., 2011). Thus, the increase of cooking loss in pasta fortified with SFP may be due to the introduction of non-gluten proteins that diluted and weakened the protein gluten network. The weakening of the pasta structure resulted in leaching of more solid material into the cooking water. Similar effects on increasing cooking losses have been reported for pasta fortified with non-durum ingredients such as seaweed (Prabhasankar et al., 2009), shrimp meat (Kadam & Prabhasankar, 2012), green mussel (Vijaykrishnaraj et al., 2014), freeze dried shrimp meat (Ramya et al., 2015), *Vicia faba* flour (Tazart et al., 2016), quinoa flour (Gimenez et al., 2016), faba bean (Rosa-Sibakov et al., 2016), almond flour (Martínez et al., 2017) & soy protein concentrate (Phongthai, D'Amico et al., 2017).

The optimal cooking time (OCT) of pasta samples ranged from 6.30 to 8.30 min (Table 2). Control pasta had the lowest lower OCT at 6.30 min. Increasing the amount of SFP in pasta from 5 to 20 %, decreased the OCT. The optimum cooking time depends primarily on the rates of water penetration and starch gelatinisation (Giuberti et al., 2015), thus the increase in the OCT of fortified pasta may due to the formation of a more complex protein network and amylose- lipid complex that may limit the water penetration into the starch granule (Moura et al., 2016). Baiano et al. (2011) reported OCT was increased with increasing amounts of soy flour incorporated into the pasta; as the soy protein is hydrophilic, it forms a gel during cooking, which then prevented the

starch from absorbing the water and gelatinising. Similarly, Ramya et al. (2015), Kadam and Prabhasankar (2012) and Liu et al. (2016) also reported that pasta fortified with shrimp meat, seaweed and meat respectively had longer cooking time than the control pasta.

The swelling index of pasta samples are presented in Table 6.2. Swelling index decreased with addition of SFP in pasta samples. In particular, swelling index of pasta significantly decreased as the inclusion of SFP increased. The reduced swelling index could be due to the formation of a protein network and starch-lipid complex in the pasta enriched with salmon powder resulting in the limited supply of water for starch granule for swelling and gelatinisation. Similar results were observed by Liu et al. (2016) who reported that swelling index decreased significantly ($P < 0.05$) as the levels of meat emulsion (15-45 g/100 g) increased in fortified pasta. Wang et al. (2016) reported that formation of starch-lipid complexes prevents leaching of amylose from starch granules during gelatinisation and inhibits swelling index. However, some research has shown a significant increase in the swelling index at increasing concentration of dietary fibre and legumes in pasta (Aravind, Sissons, Egan, et al., 2012a; Brennan & Tudorica, 2007; Cleary & Brennan, 2006; Foschia et al., 2015a; Wójtowicz & Mościcki, 2014). The difference between optimal cooking time and swelling index results obtained in this study and those reported in literature could be due to different ingredients and different processes used (Noni & Pagani, 2010).

The water absorption index is a measure of the amount of water absorbed by the pasta. The ability of pasta to absorb water is influenced by composition of raw material and processing condition (Marti, Fongaro, Rossi, Lucisano, & Ambrogina Pagani, 2011). WAI is considered to be one of the most important parameter for pasta. Table 6.2 illustrates

that the fortification of pasta with 5-20 g/100g SFP caused a significant decrease in water absorption index compared with control. The water absorption value ranged from 103.47 g/100g to 81.62 g/100 g for pasta containing 5-20 g/100g SFP respectively and was 105.46 g/100g for control. This is probably due to the substitution of semolina flour with salmon powder in pasta samples, which reduces starch swelling and pasta water absorption by formation of weak protein network through starch–lipid complex and competing with the starch for water. The highest WAI was for the control pasta due to a higher amount of starch and no lipid content. During pasta formation, it is likely that SFP competed with starch causing a reduction of starch swelling and less water absorbed by pasta containing SFP. These results are in agreement with (Bagdi et al., 2016) and Lorusso et al. (2017) who reported that pasta enriched with aleurone rich flour (protein 26.67% and fat 4.26%) and quinoa flour has significantly decreased WAI compared to wheat only pasta. Similarly, Ramya et al. (2015) and Vijaykrishnaraj et al. (2014) reported that the addition of shrimp meat and green mussel powder to pasta significantly ($P < 0.05$) decreased WAI as the level of shrimp meat and green mussel powder increased. In contrast to this study, Devi et al. (2013) reported an increase in WAI of pasta containing 15 g/100g fish mince. The higher water absorption index values obtained for pasta containing fish mince may be explained by the higher capacity of the fish mince to absorb and retain water within a very well developed starch-protein network. Recently, Tazart et al. (2015) and Laleg et al. (2016) reported that the inclusion of broad bean flour and egg into pasta caused a significant decrease in WAI compared to a wheat only. Brennan et al. (2004) reported that the WAI of pasta increased due to the increased degree of starch gelatinisation and disruption of the protein-starch matrix within the product. In this study, the results of the water

absorption indicated that starch in the pasta enriched with salmon powder may be less gelatinised due to the starch-lipid inreaction during cooking compared to the control pasta.

6.3.3 Colour measurements

Generally, consumers like pasta with a bright yellow colour (Pongpichaiudom & Songsermpong, 2018). Colour of pasta without additives depends on the properties of flour used such as carotenoids and composition of proteins (Ohm, Ross, Peterson, & Ong, 2008). Therefore, maintaining colour within consumer acceptance levels is essential. Table 6.3 and Figure 6.1 shows the L^* , a^* and b^* values for all pasta samples before and after cooking.

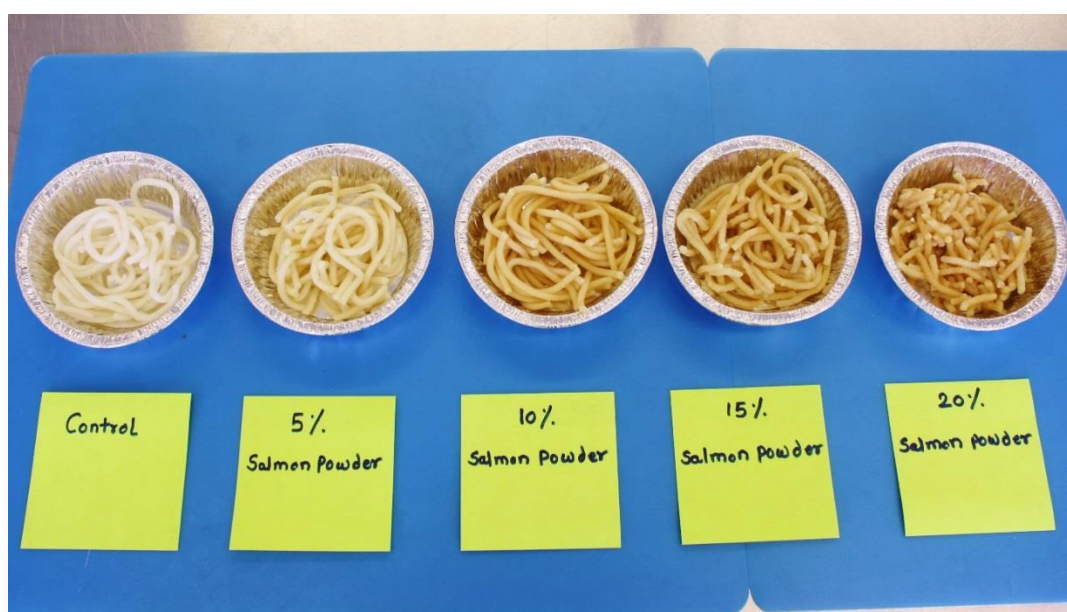


Figure 6.1 Pasta enriched different levels (5, 10, 15 and 20 %) of salmon powder.

In uncooked pasta, SFP strongly influenced the pasta colour properties and caused a decrease slightly in the lightness (L^*) from 93.97 to 94.42 compared to the control (95.58). Similarly, in case of cooked pasta, lightness decreased to between 88.18 - 91.01 compared to control (95.45).

Table 6.3 Colour characteristics of control and salmon fish powder (SFP) enriched pasta products.

Samples	Measurements			
	L^*	a^*	b^*	ΔE
Uncooked pasta				
CP	95.58 ± 0.19^a	-11.28 ± 0.33^b	28.10 ± 1.00^d	
SFP 5	94.15 ± 0.10^{abc}	-11.47 ± 0.16^b	31.82 ± 0.26^c	3.49 ± 1.08^c
SFP 10	93.97 ± 0.66^c	-9.66 ± 0.39^a	34.68 ± 0.49^b	6.30 ± 0.32^b
SFP 15	94.42 ± 0.12^{ab}	-9.03 ± 0.24^a	35.54 ± 0.07^b	7.09 ± 0.86^b
SFP 20	94.19 ± 0.29^{bc}	-8.79 ± 0.52^a	36.83 ± 0.27^a	9.59 ± 1.04^a
Cooked pasta				
CP	95.45 ± 0.24^a	-12.34 ± 0.23^d	30.11 ± 0.37^a	
SFP 5	91.01 ± 0.08^b	-11.59 ± 0.66^c	27.02 ± 1.20^c	5.63 ± 0.24^b
SFP 10	88.18 ± 0.83^c	-10.86 ± 0.23^b	26.14 ± 0.63^c	8.40 ± 0.09^a
SFP 15	87.79 ± 0.65^c	-9.57 ± 0.19^a	27.68 ± 0.53^b	8.50 ± 0.52^a
SFP 20	88.18 ± 0.48^c	-9.29 ± 0.85^a	28.45 ± 0.51^b	8.05 ± 0.65^a

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The lightness of pasta samples decreased as the amount of salmon powder in the recipe increased. This observation was more evident for cooked pasta with addition of salmon powder ($P < 0.05$). Similarly, Kadam and Prabhasankar (2012) studied the effect of shrimp meat on the colour characteristics of pasta and reported that the addition of 10-30 g/100g shrimp meat into pasta decreased the lightness (L^*) value compared to control pasta. In addition, Vijaykrishnaraj et al. (2014), Liu et al. (2016) and Phongthai et al. (2017) found that increased levels of green mussel powder, meat and egg albumen respectively in pasta showed decreased lightness. A significant ($P < 0.05$) increase in the redness parameter (a^*) of pasta enriched with salmon powder was also observed in both cooked and uncooked pasta (Table 6.3). The higher redness and corresponding decrease in lightness of colour of pasta were probably due to the

astaxanthin carotenoid pigment present in the salmon (Ortiz et al., 2013). These results were in agreement with those from Kadam and Prabhasankar (2012), Liu et al. (2016), Tazart et al. (2016) and Vijaykrishnaraj et al. (2014) who reported an increase in the red colour of pasta associated with the inclusion level of green mussel powder, vicia faba, meat and shrimp meat respectively.

The yellowness (b^*) value of pasta was compared to understand the acceptability of product. The b^* values for uncooked and cooked pasta samples were 28.10 to 36.83 and 30.11 to 28.45, respectively. In uncooked pasta, yellowness was increased significantly ($P < 0.05$) with increasing level of SFP while in cooked pasta, yellowness of pasta sample decreased significantly ($P < 0.05$) compared to control pasta. The decrease in the degree of yellowness in cooked SFP enriched pasta could be related to degradation and dissolution of carotenoid pigment by hot water, as already observed in broad bean and legume fortified pasta (Tazrat et al., 2015; Bouasla et al., 2017). Also, Gull et al. (2015) demonstrated that incorporation of millet flour and carrot pomace were significantly reduced yellowness (b^*) characteristics to those of control. The ΔE values were also determined to evaluate the colour differences between the control and the salmon powder containing formulations. The ΔE values of salmon powder containing pasta increased significantly ($P < 0.05$) with increasing levels of salmon powder in both cooked and uncooked forms (Table 6.3). In addition, uncooked pasta showed higher ΔE with increasing level of SFP in pasta, indicative of high amount of the colour compound. The ΔE values were more than 3.0 and below 12.0 for cooked and uncooked pasta. Khan et al. (2014) reported that sorghum flour enriched pasta showed higher ΔE with increasing level 20 % to 40 %.

6.4 Textural measurements

From the consumer point of view, firmness and tensile (breaking) strength are good indicators to ensure the acceptance of products and are presented in Table 6.4 and Figure 6.2. The textural properties of pasta are mainly controlled by a gluten network, which is a structural network of starches, protein, lipid additions, and other ingredients (Chang & Wu, 2008).

Table 6.4 Textural properties of enriched pasta with salmon fish powder (SFP).

Samples	Measurements	
	Firmness Peak force (N)	Tensile (breaking) strength (N)
CP	3.13 ± 0.13^a	0.48 ± 0.01^b
SFP 5	1.24 ± 0.03^d	0.54 ± 0.02^a
SFP 10	1.16 ± 0.02^d	0.51 ± 0.01^{ab}
SFP 15	2.10 ± 0.03^b	0.49 ± 0.01^b
SFP 20	1.52 ± 0.05^c	ND

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

ND: not detected

Firmness is a reflection of the bond strength and the integrity of the protein matrix present in the pasta after the cooking process (Larrosa, Lorenzo, Zaritzky, & Califano, 2016). The decrease ($P < 0.05$) in firmness of pasta was observed as inclusion SFP level increased. The firmness decreased from 3.13 N in control sample to 1.16 to 2.1 in the 5-20 % SFP sample respectively.

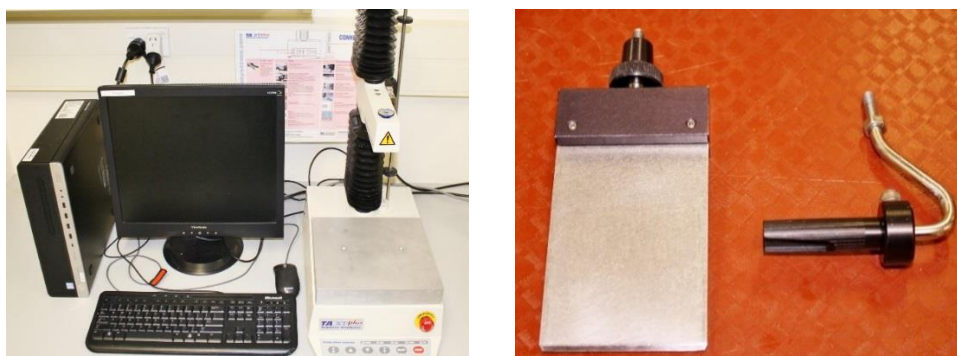


Figure 6.2 Firmness and tensile strength measurement by texture analyser.

This might be due to high cooking loss in enriched pasta that was mainly caused by their lipid content. It was also confirmed by statistically significant ($P < 0.05$) differences in lipid content of SFP enriched cooked pasta (Table 6.4). Gallegos-Infante et al. (2010) reported that the addition of common bean flour in starch matrix reduced the firmness and increase the cooking loss of the pasta. Oyeyinka et al. (2017) found that inclusion of stearic acid and linoleic acid into bambara and potato starch resulted a reduction of firmness due to the amylose in starch interacting with the lipids to form inclusion complexes which prevented the formation of double helices and protein gel network. The effect of lipid addition on mung bean starch has been investigated by Sun et al. (2013) and a 45% reduction in the firmness of starch gel paste was obtained with 6% lipid addition. Pal et al. (2017) reported that the firmness of noodles was significantly correlated to cooking loss. These relations are furthered explained by the weakening of the overall pasta structure due to the interference of starch granules by lipids and inhibition of the solubilisation of starch and the subsequent disturbance of the starch-protein matrix, as reported by (Lu, Guo, & Zhang, 2009). The textural properties of pasta in this study correspond to results from studies carried out on the addition of surimi powder and green mussel powder into pasta (Chin et al., 2012; Vijaykrishnaraj et al.,

2014) and incorporation of common bean flour, bean flour and amaranth into pasta (Gallegos-Infante et al., 2010; Giuberti et al., 2015; Islas-Rubio, Calderón de la Barca, Cabrera-Chávez, Cota-Gastélum, & Beta, 2014). Tensile (breaking) strength was examined as maximum force applied before the pasta broke (Chang & Wu, 2008). The extensibility value in this study ranged from 0.48 N to 0.54 N. This suggested that the breaking strength of pasta was improved with a reduced level of SFP level but excess addition of SFP were detrimental to noodle elasticity. This may be attributed to the higher amount of lipid content which disturb and weaken the gluten network. Lu et al. (2009) who found that incorporation lipid more than 1.25g/100g flour into noodle significantly decreased the extensibility.

6.5 Conclusion

The enrichment of pasta with SFP resulted in significantly higher protein, lipid, ash and energy contents compared with the control pasta. Cooking loss and optimal cooking time were increased with the addition of SFP to pasta. In addition, lower water absorption index, swelling index, firmness and increased extensibility were observed in pasta fortified with SFP. The addition of SFP, reduced the starch gelatinisation with starch-lipid and protein-lipid complex and effect on high cooking time. Further, weakening of the protein gluten network and thus increased cooking loss and decreased firmness and tensile strength. Colour parameters indicated that pasta were slightly darker, redder and less yellow when compared to control pasta. The results showed that inclusion of SFP up to 15 % could be a beneficial additive for pasta production with enhanced physicochemical properties creating a product with potentially beneficial

effects on human health. Further investigation of sensory features (odour, taste) of reformulated products and shelf life of the end product is required.

Chapter 7

Effect of salmon (*O. tshawytscha*) powder on starch and protein digestibility profile and antioxidant potential of semolina based pasta

This chapter is accepted for published in “**Molecules**” Journal.

Ajay S. Desai., Margaret A. Brennan., & Charles S. Brennan. Effect of salmon (*O. tshawytscha*) powder on starch and protein digestibility profile and antioxidant potential of semolina based pasta.

Abstract

This chapter reports on the effect of salmon fish (*O. tshawytscha*) powder (SFP) inclusion from 5% to 20% (w/w) on starch digestibility, protein digestibility and nutraceutical quality (phenolic content and antioxidant activity). The starch digestibility of pasta decreased significantly ($P < 0.05$) as SFP levels increased with glycaemic measurements ranging between 505-382 mg/g (108 to 143% lower than control) and the values for the glycaemic area under curve (AUC) 385-261 for 5-20% of supplemented pasta, respectively. Enrichment with SFP affected the *in vitro* bioaccessibility of nutrients. The phenolic bio-accessible fraction of pasta enriched with 20% SFP was 179% (gastric) - 133% (pancreatic) increased compared to the control pasta, and the antioxidant activity was 263% (gastric)-190% (pancreatic) higher. Supplementation of SFP reduced protein digestibility (86.41% for control pasta; 81.9 % for 20% SFP pasta). The associated decrease in amino acid content confirmed the presence of interactions between phenolic and protein. Overall, the results showed that

inclusion SFP is a promising way to mitigate the starch digestibility and increased antioxidant activity in pasta product.

7.1 Introduction

Salmon (*Oncorhynchus tshawytscha*) is the main aquaculture species in New Zealand. It is rich in long chain ω -3 polyunsaturated fatty acids (LC ω -3PUFAs) namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These compounds are not synthesised by the human body and need to be ingested as part of a healthy diet from external source with diet (Ortiz et al., 2013). A high intake of LC ω -3PUFAs has beneficial effects in human health including reducing the impact of cardiovascular diseases, diabetes, cancer, obesity, asthma and depression (Cascant et al., 2018). Fish has been shown to be an important source of nutrients such as vitamins (A, D, B6 and B12) and minerals (iron, zinc, iodine, selenium, potassium and sodium (Matos et al., 2015). The American heart association (AHA) recommends a minimum of two fish servings per week (approximately 200mg/day of long chain ω -3 polyunsaturated fatty acid (PUFA)) to achieve the cardio protective effect, however intake is below this in most countries. Fish powder is a by-product of fish processing and represents a cheap source of high quality nutrients (protein and lipid) which may be utilised in the human diet (Oliveira, Lourencno, Sousa, Peixoto Joele, & Ribeiro, 2015). Salmon is also a good source of antioxidant compounds such as astaxanthin carotenoids (Ortiz et al., 2013). Consumption of antioxidant rich food products has been related to a decreased in diseases related to oxidative stress (Pisoschi & Pop, 2015). Fish powder is a protein rich material which may contribute to a low glycaemic index and have beneficial health effects such as the manipulation of obesity, hypertension and cardiovascular disease in

human beings (Kadam & Prabhasankar, 2010). Both consumers and the scientific community have a raised interest in healthy food ingredients which may have high levels of protein and lipid (such as fish protein) and alter the nutritional quality of foods such as glycaemic response, essential amino acids, protein digestibility and phenolic compounds (Brennan et al., 2013). To achieve this, pasta products have been fortified with high protein sources, such as soya flours, soy isolates, milk and milk products, whey proteins, yeast protein concentrates and meat (Liu et al., 2016; Torres et al., 2006).

Pasta is a popular starch based stable food, containing (74–77%) carbohydrates, and is regarded as a low glycaemic index food. It is consumed widely worldwide and is second to bread as the most consumed cereal food product (Tazart et al., 2016). Starch, protein and lipid are the three major macromolecules in cereal based pasta products and play important role in human nutrition such as the glycaemic response. Consumption of starchy food with high amounts of rapidly digestible starch is associated with the development of blood glucose level and may be related to obesity and being overweight (Chen et al., 2017a). Foods with a low glycaemic value could avoid the outbreak of diabetes, cardiovascular and neurodegenerative disease (Augustin et al., 2015). The rate of starch digestion, and glycaemic response, have been shown to be related to the presence of proteins (Chen et al., 2017b), the amount and type of dietary fibre (Foschia et al., 2015a), and the presence of lipids (Chen et al., 2017a). The ability to form amylose-lipid complexes in starch based products is well known. The complex development between amylose and lipid has been used to prepare pasta products. In addition, complexing with lipids confers resistance on amylose to amylase hydrolysis by digestive enzymes (Oyeyinka et al., 2017). Previously researchers have studied the effects of different food lipids, including butter, coconut oil, grapeseed oil and olive oil

of different degree of saturation and chain lengths, on the glycaemic response of bread (Lau et al., 2016). Lipids significantly decreased the starch hydrolysis rate, and the formation amylose-lipid complexes and protein-lipid complexes may be responsible for this observation (Chen et al., 2017a). The presence of protein in the food matrix may influence starch digestion by the encapsulation of starch granules into the protein matrix of the food (Brennan et al., 2013). The effect of meat protein interactions on the digestibility of pasta has been studied (Liu et al., 2016). The researchers observed that starch-protein interactions increased with increasing levels of meat additions and accounted for decreasing glycaemic responses. Also, interaction between starch-protein- phenolic compound in the food product affect protein structure through precipitation and decrease the starch and protein digestibility (Czubinski & Dwiecki, 2017). The supplementation of pasta with other functional ingredients has received much attention. For instance pasta has been fortified with protein rich ingredients such as faba bean flour (Coda et al., 2017), meat (Liu et al., 2016), shrimp powder (Ramya et al., 2015), green mussel powder (*Perna canaliculus*) (Vijaykrishnaraj et al., 2014), barely flour (Montalbano et al., 2016) and amaranth seed flour (Cardenas-Hernandez et al., 2016). However, the nutritional properties of pasta enriched with partial replacement of semolina wheat flour by salmon (*Oncorhynchus tshawytscha*) powder (SFP) is still unknown. Therefore, the present investigation aimed to evaluate the effects of salmon powder as ingredients for pasta production and their contribution to *in vitro* starch, protein digestibility and antioxidant activity.

7.2 Materials and methods

7.2.1 Raw materials

Described in section 3.1

7.2.2 Salmon powder preparation

Described in section 3.1.1

7.2.3 Pasta production

Described in section 3.2.2

7.2.4 *In Vitro* starch digestibility and glycaemic response

Described in section 3.4.6

7.2.5 *In vitro* protein digestibility

Described in section 3.4.5

7.2.6 Amino acid profile of digesta

Described in section 3.4.7

7.2.7 Total phenolic content (TPC)

Described in section 3.4.12.1

7.2.8 Antioxidant activity (Oxygen Radical Absorbance Capacity, ORAC Assay)

Described in section 3.4.12.2

7.2.9 Sensory analysis

Described in section 3.4.14

7.2.10 Statistical analysis

Described in section 3.4.22

7.3 Results and discussion

7.3.1 *In Vitro* starch digestibility and glycaemic response

The digestibility of pasta products plays an important role in human nutrition. Protein, lipid and starch are three major components in pasta products. The interactions among them play an important role in starch digestibility and further predictive glycaemic

response in the human small intestine (Ren et al., 2016). This study investigated the effects of lipid and protein on *in vitro* starch digestion characteristics of pasta enriched with SFP. The effect of supplementation of SFP were studied by measuring the amount of reducing sugars released over 120 min *in vitro* digestion. Figure 7.1 shows the amount of reducing sugars released over 120 min *in vitro* digestion of pasta samples. The enrichment of pasta with SFP resulted in reduced reducing sugar levels ($P < 0.05$) compared to the control. The proportion of rapidly digestible starch (RDS) and slowly digestible starch (SDS) fraction in pasta enriched with SFP are presented in Figure 7.2.

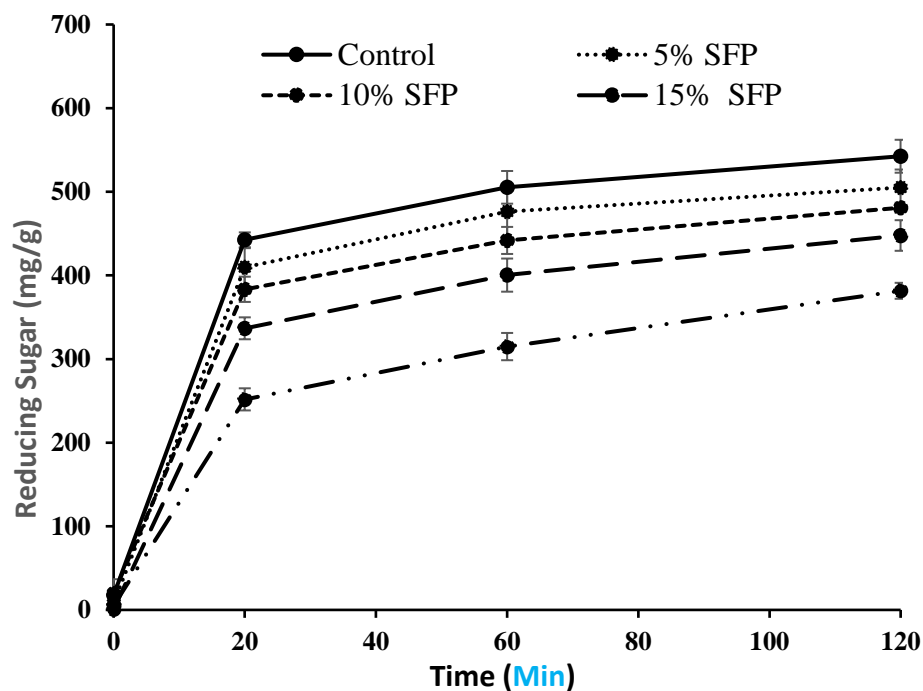


Figure 7.1 Amount of reducing sugar released during *in vitro* digestion for control (C), and pasta containing 5% salmon fish powder (SFP), 10% SFP, 15 % SFP and 20% SFP respectively.

All pasta samples enriched with SFP had significantly lower levels of RDS and SDS than the control pasta ($P < 0.05$), possibly due to fact that the lipids formed complexes with amylose and protein blocked enzyme adsorption sites on the surface of starch granule (Chen et al., 2017a).

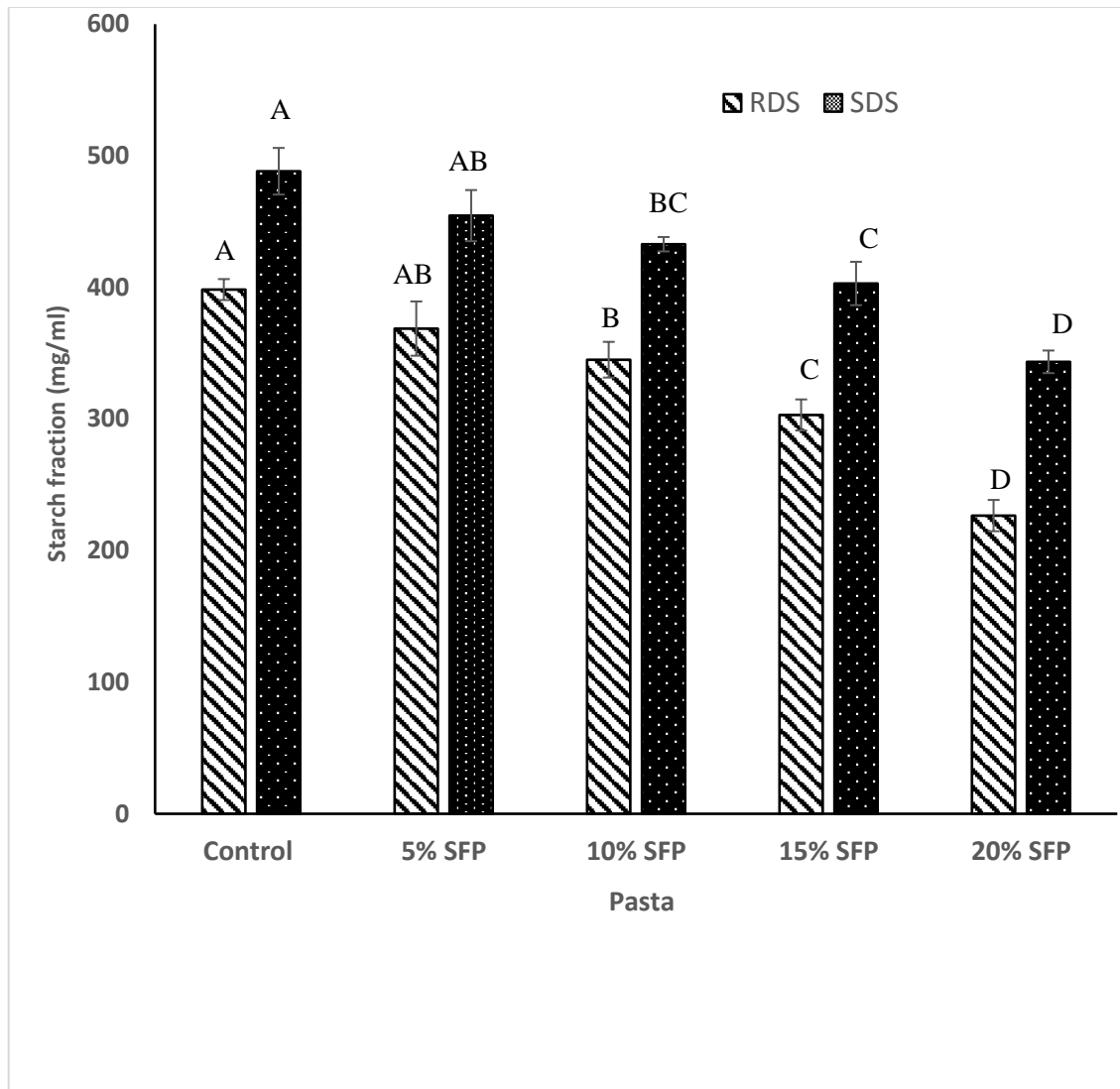


Figure 7.2 Starch content hydrolysed within 20 min readily digestible starch (RDS) left and within 120 min slowly digestible starch (SDS) right of pasta enriched with 5%, 10 %, 15% and 20% SFP. The values are expressed as mean \pm SD (n=3). Different letters showed the significant difference ($P < 0.05$).

The addition of protein rich bean flour into spaghetti has been shown to significantly lower the RDS and SDS fractions when compared to semolina spaghetti (Giuberti et al., 2015), possibly due to incomplete gelatinisation of starch granules. In the present study, cooked pasta samples enriched with 5% SFP, 10% SFP, 15% SFP and 20% SFP had 0.25%, 1.25%, 2.59% and 3.69% lipid content, respectively. The reduction in digestibility may be attributed to the formation of amylose-lipid complexes (ALC). Previous research has indicated that ALC has enzymatic resistance which increases with increasing lipid chain

length (Ren et al., 2016). Lipids can also interact with amylose and prevents starch granules hydration and swelling. The amylose-lipid interactions results in the formation of single helical structure with a conformational hindrance that restricts enzymes to hydrolyse the starch granule. This phenomenon has been confirmed by adding different fats to baked bread, which result in a significant reduction of *in vitro* starch digestibility with formation of amylose-lipid complex (Lau et al., 2016). Several studies have aimed to investigate the interaction of lipid and starch and its effect on *in vitro* digestion (Annor et al., 2015; Oyeyinka et al., 2017; Ren et al., 2016). For instance when stearic acid and linoleic acid were incorporated into bambara starch a decrease in starch digestibility was observed associated with the formation of single helical structure of amylose-lipid complex (Oyeyinka et al., 2017). Similar results were reported when investigating the *in vitro* starch digestibility and glycaemic index of millets made with different fatty acids palmitic, oleic and linoleic acids (Annor et al., 2015). Additionally, proteins reduced starch granule surface accessibility and therefore influenced the enzyme susceptibility. In our study, protein rich SFP was used and this may also contribute the development of a strong protein network which entrap the α -amylase (Singh, Dartois, & Kaur, 2010). Several researchers have studied the effect of addition protein rich ingredient into pasta on starch digestibility (Liu et al., 2016; Ramya et al., 2015), and illustrated that the addition of SFP may encapsulated starch molecules and reduced the starch granules surface accessibility of α -amylase to starch. It was previously reported that the presence of protein content in food matrix creates a strong network and reduces the capacity of enzyme attack to the starch granules, thereby delaying starch digestion (Rosa-Sibakov et al., 2016).

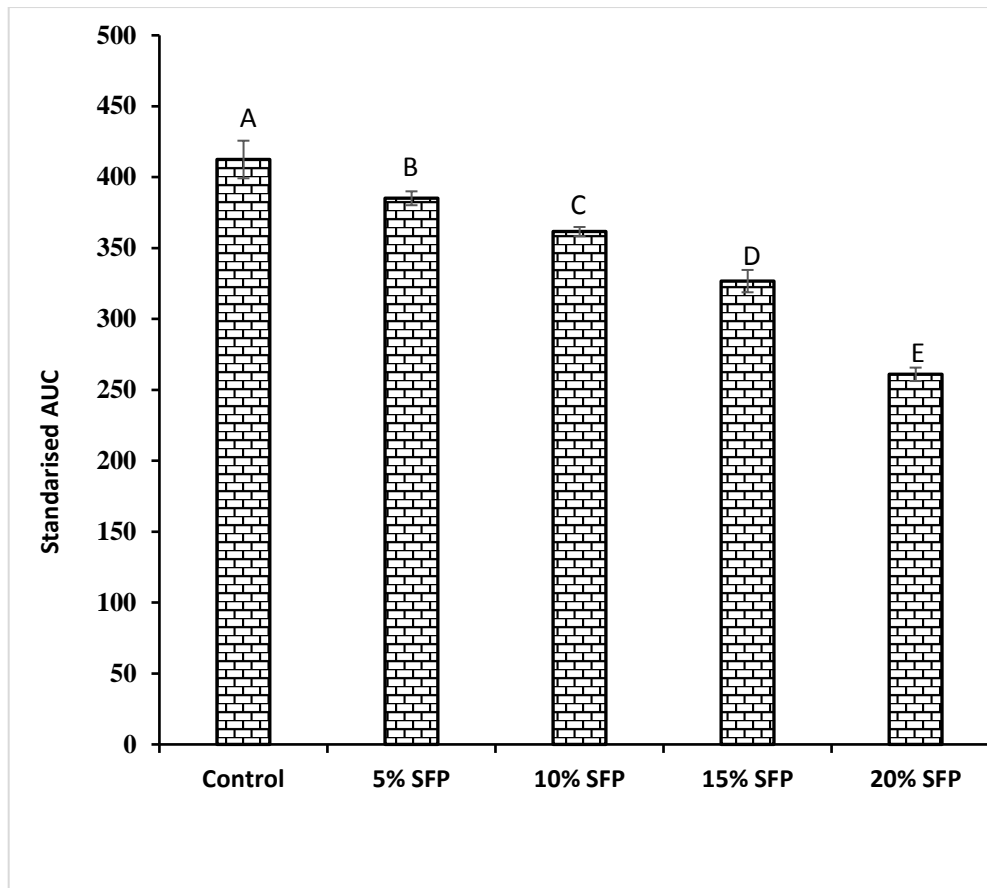


Figure 7.3 Values for area under the curve (AUC) comparing control and enriched salmon fish powder (SFP) pasta samples.

A similar effect on starch digestibility has been observed with the addition of yam flour (*Dioscorea schimperiana*) into wheat pasta (Djeukeu et al., 2017), where the yam flour formed protein matrix as a physical barrier around the starch granules and protected them from enzyme attack. Figure 7.3 illustrates the effects of substituting semolina flour with SFP on standardised AUC values as comparison against the control (100% durum wheat semolina) sample. In all samples, AUC values were significantly ($P < 0.05$) decreased with increasing levels of SFP into pasta. In particular, Previous research reported the development of spaghetti by replacing semolina with legume soya bean flour and resulted in significant ($P < 0.05$) reduction in AUC values compared to those of control spaghetti with semolina and this indicated that soya bean flour can decrease

carbohydrate intake effect on lower glycaemic response (Chillo et al., 2010). Based on these results, it could be concluded that the SFP enriched pasta was more resistant to digestion compared to the control pasta without SFP. The addition of SFP into pasta like product is a convenient and novel choice in lowering the glycaemic index of the final product.

7.4 Protein and *In vitro* protein digestibility

The *in vitro* protein digestibility gives information on the stability of protein to digestive process. The quality of protein is one of the most important attributes to determine the nutritional characteristics of a food matrix and is normally evaluated by protein digestibility (Tazart et al., 2016). Table 7.1 shows the values of *in vitro* protein digestibility, protein content in cooked and uncooked pasta, and protein availability after digestion. No previous information was available about the *in vitro* digestibility of pasta enriched with SFP.

Table 7.1 Protein content, *In vitro* protein digestibility and protein availability of pasta fortified with salmon fish powder (SFP).

Samples	PC in raw pasta (g/100g dry pasta)	PC in cooked pasta (g/100g dry pasta)	PD (%)	PA (g/100g dry pasta)
CP	12.60 ± 0.05 ^a	12.88 ± 0.06 ^a	86.41 ± 0.37 ^a	11.13 ± 0.07 ^a
SFP 5	14.34 ± 0.03 ^b	15.41 ± 0.17 ^b	84.60 ± 0.20 ^b	13.03 ± 0.14 ^b
SFP 10	17.67 ± 0.04 ^c	18.10 ± 0.11 ^c	82.97 ± 0.10 ^c	15.02 ± 0.09 ^c
SFP 15	20.73 ± 0.10 ^d	20.77 ± 0.09 ^d	81.16 ± 0.27 ^d	16.85 ± 0.12 ^d
SFP 20	22.7 ± 0.30 ^e	23.40 ± 0.13 ^e	81.95 ± 0.18 ^e	19.18 ± 0.07 ^e

PC- protein content, PD- *In vitro* protein digestibility, PA-protein availability. SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta. Results are presented as the mean value ± standard deviation, $n = 3$; Values within a column followed by different small letters are significantly different ($P < 0.05$).

Table 7.1 illustrates that the addition of SFP resulted in a significant ($P < 0.05$) increase of protein content of pasta samples however, no significance difference ($P > 0.05$) were

observed between uncooked and cooked pasta, indicating that the protein did not leach out of the pasta during the cooking process. The percentage of *in vitro* protein digestibility of pasta enriched with SFP was significantly ($P < 0.05$) reduced from 84.60 to 80.80% as a result of the increase in the level of the SFP. The reduction in digestibility could be due to fish protein structure, other components such as formation of protein-starch complex, cross links between proteins (Giménez et al., 2016) and presence of phenolic compounds (Swieca et al., 2014).

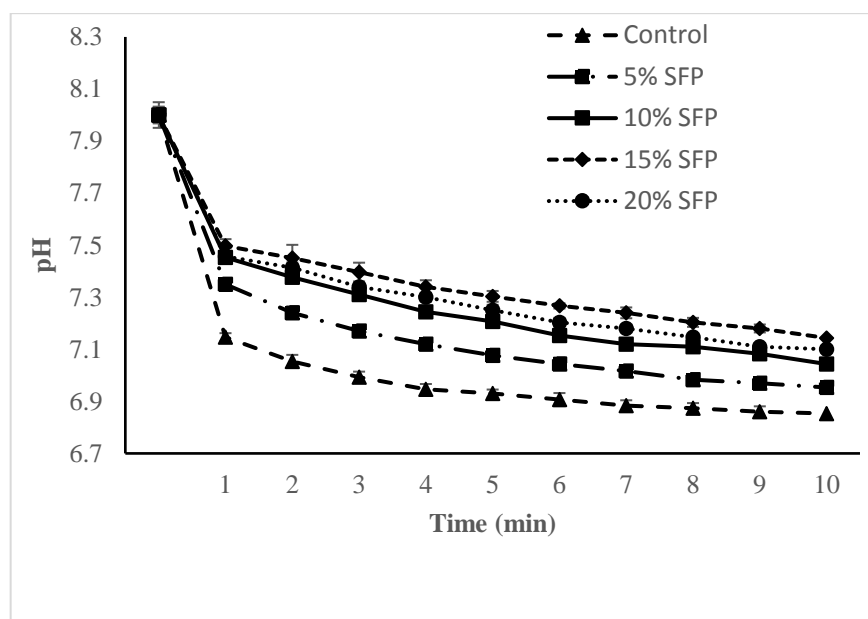


Figure 7.4 The pH vs time curves obtained by pasta made with different concentration of salmon fish powder (SFP) incubated with multi-enzymes (Trypsin, Chymotrypsin and protease).

Oxidized phenolic compounds have been proposed to react with proteins and form insoluble complexes, inhibiting the activity of proteolytic enzymes and interfering with utilisation of proteins (Ozdalet al., 2013). Our results are supported by those previously reported Kadam & Prabhasankar (2012), which found a reduction of protein digestibility of shrimp meat and broad bean enriched pasta. The pH drop curves obtained from proteolytic enzymatic hydrolysis of enriched pasta with SFP exposed to standardised

mixture of commercial purified enzymes (trypsin, α -chymotrypsin and peptidase) are shown in Figure 7.4. These curves also exhibited a drop in pH value with time. The drop in pH results from the release of amino acids and peptides, protein building units, as protein are digested and as carboxyl ($-\text{COO}^-$) and amino ($-\text{NH}_3^+$) units are released. At neutral pH (8.0), the free amino groups deionize in water and protons (H^+) are liberated. These free H^+ released into the surrounding reaction medium cause as decrease in pH. At alkaline pH, phenolic compound present in enriched pasta could be oxidised by oxygen with side amino groups of peptides to form quinines and this lead to formation of protein cross-links. These quinines react with sulfhydryl and amino groups of proteins, result into decreased protein digestibility (Prodpran et al., 2012).

7.5 The composition of amino acids released into intestine after *in vitro* digestion

The quality of proteins is assessed through their breakdown of amino acids in the small intestine. The protein digestibility in combination with amino acid composition released in the small intestinal stage therefore gives a better prediction of the nutritional value of pasta. After 2 h of digestion in the small intestine, amino acids released into the intestine are ready to be used by the body. In this study, amino acid content of pasta enriched with SFP and control after 2 h digestion is shown in Table 7.2. The phenylalanine, tyrosine, isoleucine and leucine content of enriched pasta decreased significantly ($P < 0.05$) compared to the control while no significant difference were noticed in the methionine, threonine, tryptophan and valine amino acids. The addition of SFP also significantly ($P < 0.05$) decreased non-essential amino acids in the digesta except arginine, alanine and asparagine compared to control pasta.

Table 7.2 Amino acid (AAs) composition (mg/g protein) from digestibility studies in the intestinal stage at 120 min of cooked pasta enriched with different salmon fish powder (SFP) levels and control.

Amino acid	CP	SFP5	SFP10	SFP15	SFP20
Phenylalanine	18.07 ± 0.17 ^a	14.63 ± 1.13 ^{bc}	14.86 ± 1.09 ^b	13.40 ± 0.46 ^{bc}	12.68 ± 0.31 ^c
Tyrosine	14.03 ± 0.37 ^a	12.07 ± 0.96 ^b	12.61 ± 1.07 ^{ab}	11.40 ± 0.30 ^b	10.90 ± 0.39 ^b
Isoleucine	14.94 ± 0.10 ^a	12.55 ± 1.05 ^a	13.48 ± 1.09 ^{ab}	12.24 ± 0.31 ^b	11.98 ± 0.23 ^b
Leucine	26.82 ± 0.21 ^a	22.72 ± 1.78 ^b	23.92 ± 1.86 ^{ab}	21.87 ± 0.60 ^b	21.68 ± 0.29 ^b
Lysine	16.15 ± 0.33 ^b	16.72 ± 1.30 ^b	21.58 ± 1.58 ^a	21.41 ± 0.58 ^a	22.27 ± 1.54 ^a
Methionine	0.57 ± 0.35 ^a	0.81 ± 0.61 ^a	0.51 ± 0.17 ^a	0.47 ± 0.15 ^a	0.46 ± 0.08 ^a
Threonine	13.14 ± 0.18 ^a	11.67 ± 0.90 ^a	13.18 ± 0.98 ^a	12.08 ± 0.38 ^a	11.96 ± 0.42 ^a
Tryptophan	6.85 ± 0.84 ^a	5.56 ± 0.96 ^a	6.05 ± 0.24 ^a	5.44 ± 0.44 ^a	5.21 ± 0.07 ^a
Valine	16.42 ± 0.55 ^a	14.09 ± 1.44 ^a	15.72 ± 1.28 ^a	14.25 ± 0.31 ^a	13.92 ± 0.58 ^a
ΣEAAAs	126.99	110.82	121.91	112.56	111.06
NEAAAs					
Arginine	21.64 ± 0.22 ^{ab}	18.13 ± 1.58 ^b	23.60 ± 1.38 ^a	21.04 ± 1.37 ^{ab}	20.44 ± 2.07 ^{ab}
Alanine	14.32 ± 0.09 ^a	13.05 ± 0.97 ^a	14.63 ± 1.10 ^a	13.71 ± 0.34 ^a	13.75 ± 0.22 ^a
Glutamic acid	85.31 ± 13.45 ^a	73.76 ± 5.11 ^{ab}	77.68 ± 5.86 ^{ab}	65.44 ± 0.92 ^{ab}	55.85 ± 4.59 ^b
Glycine	17.00 ± 0.33 ^a	14.61 ± 1.01 ^b	15.02 ± 1.22 ^{ab}	13.98 ± 0.58 ^b	13.53 ± 0.54 ^b
Proline	37.87 ± 2.08 ^a	23.43 ± 4.20 ^b	25.67 ± 2.20 ^b	21.64 ± 0.72 ^b	19.32 ± 1.90 ^b
Serine	16.15 ± 1.32 ^a	14.08 ± 1.21 ^{ab}	15.00 ± 1.22 ^{ab}	13.33 ± 0.33 ^{ab}	12.43 ± 1.00 ^b
Asparagine	20.58 ± 0.41 ^{ab}	19.50 ± 1.50 ^b	23.03 ± 1.78 ^a	21.29 ± 0.70 ^{ab}	21.19 ± 0.63 ^{ab}
ΣNEAAAs	212.87	176.56	194.63	170.43	156.51

Histidine, Aspartic acid, Cysteine, Glutamine amino acid: not detected

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Results are presented as the mean value ± standard deviation, $n = 3$; Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The decrease in amino acid availability could be due to interactions of protein and lipid

- phenolic compounds in pasta resulting in the oxidation of proteins and amino acids

rendering them unavailable to proteolytic enzyme hydrolysis and subsequent intestinal

absorption. This has been observed in a previous study focussing on the addition of

legumes to rice based extruded products (Pastor-Cavada et al., 2011).

7.6 Phenolic content and antioxidant activity

In recent years, a new trend has progressed to assess the beneficial aspects of food

matrix in terms to provide nutrients to fulfil metabolic requirements and their role in

improving human health. In this perspective, the SFP enriched pasta may result with an enhanced nutritional profile. In this study, the *in vitro* digestion process showed that pasta fortified with SFP released significant amounts of bio-accessible phenolic compound with increased antioxidant activity (Figure 7.5). Bioaccessibility is regarded as the amount of bioactive compounds released from the food matrix during digestion and available to absorption (Ma, Zhou, & Huang, 2014). Fish protein have bioactive properties, but they have not been as extensively studied as peptides from other sources such as milk (Rustad et al., 2011).

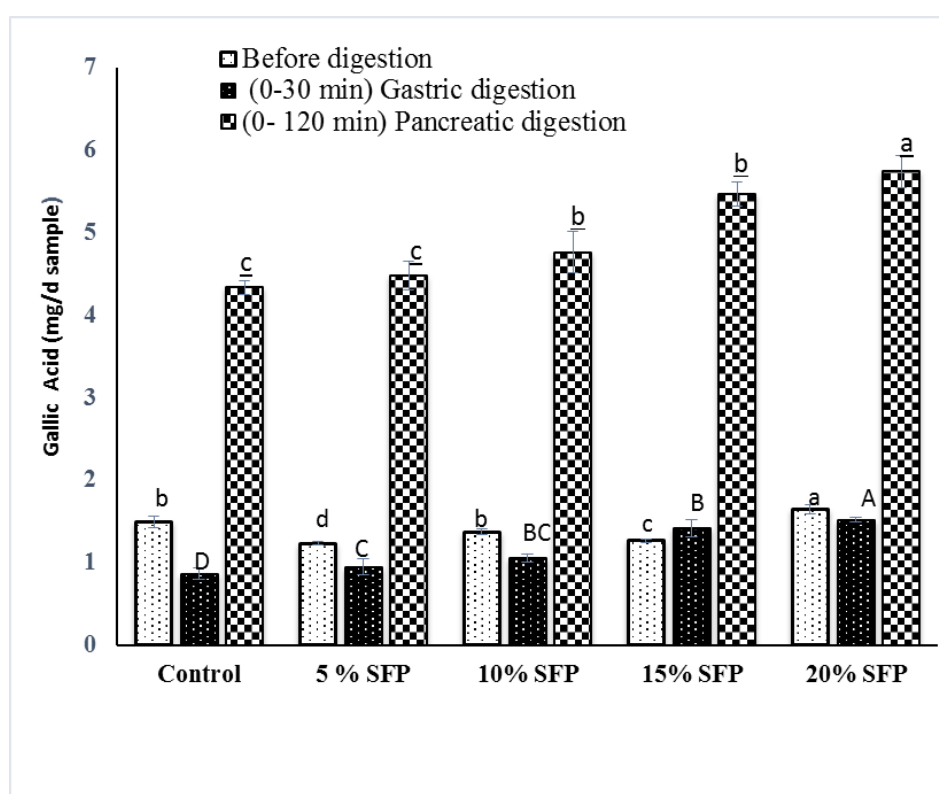


Figure 7.5 Total phenolic content of pasta enriched with different concentration of (SFP), before digestion and at gastric and pancreatic digestion. Bar represent mean \pm SD (n=3), followed by different small (before digestion), capital (gastric) and small underlined (pancreatic digestion) letters indicate significant difference among the values at $P < 0.05$.

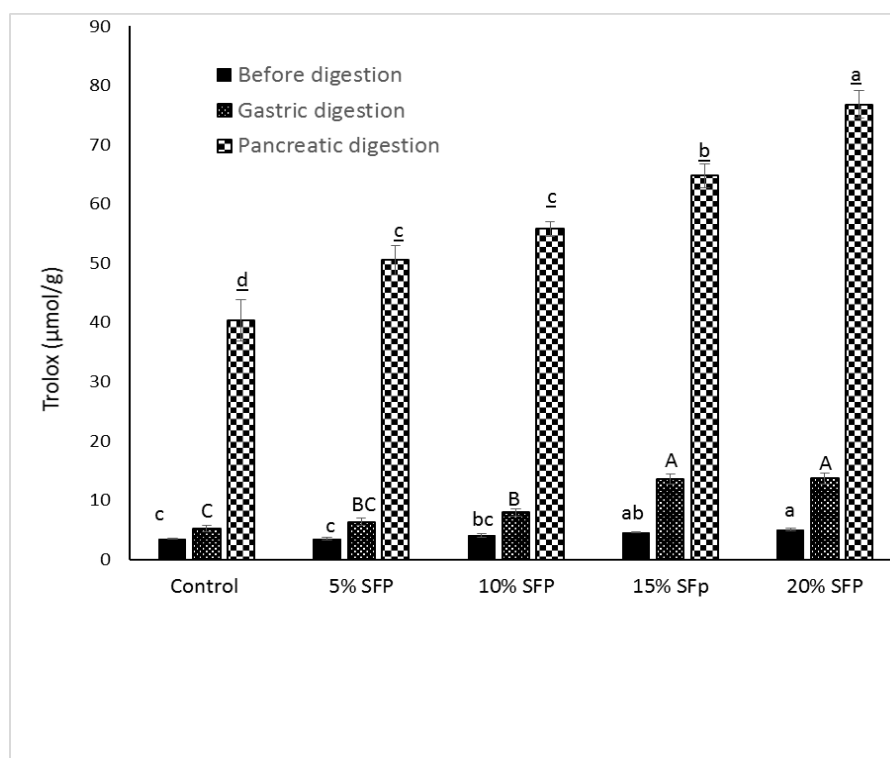


Figure 7.6 Antioxidant activity of pasta enriched with SFP determined with ORAC assay during *in vitro* gastric and pancreatic phase of digestion and before digestion. Results are expressed as Trolox ($\mu\text{mol/g}$). Data are mean \pm SD ($n=3$), followed by small (before digestion), capital (gastric) and small underlined (pancreatic digestion) letters indicate significant difference among the values at $P < 0.05$.

The results describing the effect of pasta fortification before and after digestion on the phenolic content and antioxidant activity of pasta are presented in Figures 7.5 & 7.6. Comparing the SFP pasta samples against the control pasta, after both gastric and pancreatic digestion, the amount of bio-accessible total phenolic compound in fortified pasta were significantly ($P < 0.05$) higher from 0.82 to 1.47 mg of gallic acid/g of pasta (109% to 179%) and 4.31 to 5.73 mg of gallic acid/g of pasta (103% to 133%) respectively indicating that adding SFP ingredients is an alternative to enhance its phenolic compound. Antioxidant activity was observed by oxygen radical absorbance capacity (ORAC) mechanism. An elevation from 5.20 to 13.69 $\mu\text{mol Trolox / g}$ of pasta (gastric digestion) and 40.36 to 76.75 $\mu\text{mol Trolox / g}$ of pasta (pancreatic digestion) was

observed as SFP values increased (5-20%) (Figure 7.6). The total phenolic content of the control sample before and after digestion (1.49 mg of gallic acid/g of pasta and 0.82 mg of gallic acid/g of pasta) and antioxidant activity (5.20 μ mol Trolox / g of pasta and 40.36 μ mol Trolox / g of pasta) was lower than the SFP fortified pastas. This may be attributed to cooking time and leaching of phenolic compounds from the control pasta samples into the cooking medium. A similar result has been observed, in that a significant decrease in total phenolic content was observed in cooked faba bean flour fortified pasta (Turco et al., 2016) with the phenolic compounds leaching into cooking medium and degraded due to thermal treatment. In present study pasta fortified with SFP was able to retain phenolic compound upon cooking (Swieca et al., 2013). Similar to our current study, comparable findings between phenolic level, antioxidant activity and the supplemental level of fortified pasta have been reported previously (Sęczyk et al., 2016a; Vijaykrishnaraj et al., 2014). For instance the fortification of pasta with parsley leaves at different levels (1% to 5%) increased the total phenolic content by 126-167% and antiradical activity against ABTS by 161-246% for cooked pasta (Sęczyk et al., 2016b). This finding is confirmed by previous studies concerning phenolic-protein (Swieca et al., 2013). Protein and phenol interact each other through covalent or non-covalent interaction (Ozdal et al., 2013). These interactions might lead to precipitation of protein from food matrix. For instance previous research has shown that covalent bonding may affect both secondary and tertiary structure of protein (Rawel et al., 2005). Besides the type and level of functional ingredients used, other factors such as processing and cooking are responsible for alteration of antioxidant properties of pasta (Sun-Waterhouse et al., 2013). Additionally, the presence of oxygen, water and heat

treatment during cooking and pasta making may induce the oxidation of sensitive phenolic antioxidants.

7.7 Sensory analysis of pasta products

The hedonic sensory parameters (i.e, appearance, colour, aroma, taste, texture and overall quality) of cooked pastas with different formulations are given in Figure 7.7. There were total five formulations including control with semolina and remaining pastas were fortified with 5% and 15% cod and salmon powder respectively. The results show that all the parameters were above the minimum limit for acceptability (4.5) (Silva, Gerritsen, Dekker, Van der Linden, & Scholten, 2013). Overall, the parameters 'appearance' and 'colour' did not show significant difference ($P < 0.05$) between control samples and enriched pasta samples with 5% and 15% cod and salmon powder respectively. The score obtained to appearance and colour in the pasta fortified with 5% and 15% cod and salmon powder is higher than control sample. The sensory data for colour are consistent with the instrumental colour data in which control pasta scored significantly lower ($P < 0.05$) for yellowness and brightness compared to all fortified pastas with fish powder. Panel members rated lower ($P < 0.05$) scores for taste, aroma, texture and overall quality for cod and salmon powder fortified pastas than the control pasta. For acceptability of taste, aroma, texture, and overall quality, the control pasta and pasta at 5% cod and 5% salmon powder incorporation scored in the range of "like slightly" while pasta fortified with 15% cod and 15% salmon powder scored in the range of "neither like nor dislike". The results obtained on sensory attributes in the present study are consistent with those of (Kadam & Prabhasankar, 2012; Ramya et al, 2015)

who obtained similar score for appearance, colour, aroma, taste, texture and overall quality of pasta incorporating freeze dried shrimp meat and shrimp meat.

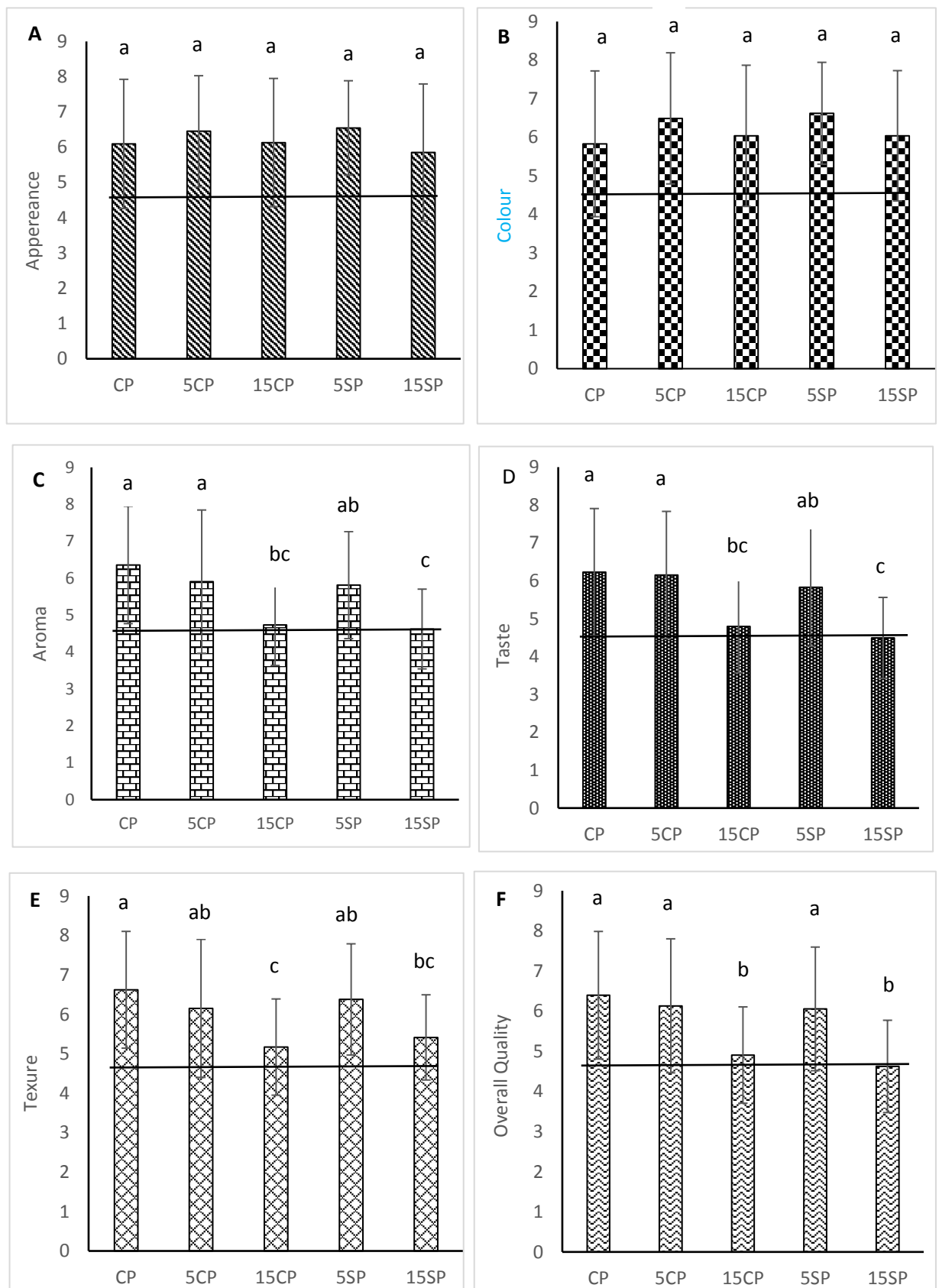


Figure 7.7 Sensory analysis, appearance (A), Colour (B), Aroma (C), Taste (D), Texture (E) and Overall quality (F) of pasta fortified with 5% and 15% cod powder (CP) and salmon powder (SP) and control. The reported values refer to the mean \pm standard deviation. The same letter above the bar indicates no significant difference between samples ($P < 0.05$) as determined with Tukey's test.

7.8 Conclusion

In conclusion, this study demonstrated that addition of SFP in pasta, is an effective ingredient to improve antioxidant capacity and starch digestibility of the final product. However, the quality of fortified pasta is affected by multiple factors, protein-starch and protein-phenol interactions. These interactions between SFP protein and semolina starch and protein influenced antioxidant activity, starch and protein digestibility. The digestibility (starch hydrolysis) of pasta was significantly reduced by incorporating SFP, however, it caused significant decreased in protein digestibility. It was proved that phenolics bind with semolina proteins and enzymes with digestive tract. Moreover, antioxidant activity from supplemented pasta are highly bio-accessible *in vitro* and significantly increased with the supplementation of SFP. In summary, to develop pasta like product with well-balanced nutritional and pro-health properties with protein rich ingredients, knowledge of the interaction existing between protein-starch-phenolic in food matrix is necessary.

Chapter 8

Amino acid and fatty acid profile and digestible indispensable amino acid score of pasta fortified with salmon (*O. tschawytscha*) powder.

This chapter is published as

Ajay S. Desai., Margaret A Brennan., & Charles S Brennan (2018). Amino acid and fatty acid profile and digestible indispensable amino acid score of pasta fortified with salmon (*O. tschawytscha*) powder. **European Food Research and Technology**, **244** (10), 1729-1739. (<https://doi.org/10.1007/s00217-018-3085-5>).

Abstract

Salmon (*O. tschawytscha*) is characterised by being rich in amino acids and long chain polyunsaturated fatty acids (PUFA) known for its health benefits. Pasta was prepared with different levels (5-20%) of salmon fish powder (SFP) and control made with semolina. Chemical composition, amino acid and fatty acid profiles of the pasta were determined. Fortification of pasta with SFP increased ($P < 0.05$) indispensable amino acid (IAA) content compared to the control. The incorporation of 5-20% SFP in the pasta formulation decreased the n6:n3 ratio from 19:1 (the control pasta) to 5:1 to 3:1 respectively. Digestible indispensable amino acid scores (DIAAS) were calculated using published data on amino acids digestibility to evaluate the protein quality of the pasta. IAA contents (mg IAA/g protein), were found to be highest in the enriched pasta with SFP (372 to 453) and lowest in the control pasta (328), all exceeded the FAO recommended daily allowance (277 mg IAA/g protein) and contributed on average 41% to total amino acid contents. The DIAAS value in the control pasta was 36 % (lysine) and

the pasta enriched with SFP containing had a DIAAS between 75 to 99%. Therefore, to fulfil the daily human AA requirement, the control pasta needs to be supplemented by amino acid and fatty acid rich salmon fish protein to achieve greater lysine concentration with DIAAS.

8.1 Introduction

Pasta has been consumed all over the world because of its low cost, ease of preparation with durum wheat semolina flour and it places second after bread (Lu et al., 2016; Tazart et al., 2016). Pasta, being a wheat based product, contains (74-77%) carbohydrate and (11-15%) protein but is deficient in limiting amino acids such as lysine and threonine (Martínez et al., 2017). Low protein content and digestibility of cereals, together with limiting essential amino acids (lysine, methionine, threonine) and fatty acids represents low nutritional value as compared with animal protein (Carbonaro et al., 2012). Consumers have increasingly demanded wheat-based products considering them to have added value. Adding exogenous ingredients rich in protein and essential amino acid (EAA) is an ideal way to get the pasta with higher protein content, a better amino acid and fatty acid pattern (Li, Zhu, Guo, Brijis, & Zhou, 2014). To improve the protein content, essential amino acid and fatty acid profile, the pasta has been supplemented with fish powder (*Nemipterus japonicus*) (Parvathy, Bindu, & Joshy, 2017), freshwater fish (Zhao, Jiang, Xu, & Xia, 2017), quinoa flour (Lorusso et al., 2017), arbinoxylans (Fan, Ma, Wang, & Zheng, 2016), flaxseed flour (Moura et al., 2016), shrimp (*Penaeus monodon*) meat (Ramya et al., 2015), tilapia (*Oreochromis niloticus*) flour (Monteiro et al., 2016), shrimp powder (*Litopenaeus vannamei*) (Jeyakumari, Rahul Das, Bindu, Joshy, & Zynudheen, 2016), protein hydrolysate (*Merluccius capensis*) (Teixeira, Pires, Nunes, & Batista, 2016), seaweed (Prabhasankar et al., 2009), carp fish

powder (*Cyprinus carpio*) (El-Beltagi et al., 2017) and blue whiting (*Micromesistius poutassou*) (Egerton, Culloty, Whooley, Stanton, & Ross, 2018). Salmon (*Oncorhynchus tshawytscha*) is the main species in marine aquaculture in New Zealand. It is rich in long chain ω -3 polyunsaturated fatty acids (LC ω -3PUFAs) namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are not synthesised by the human body and need to be ingested as part of a healthy diet from an external source (Verardo et al., 2009). A balanced ratio of ω -6/ ω -3 fatty acids promotes beneficial effects in human health including reducing the impact of cardiovascular diseases, diabetes, cancer, obesity, asthma, and depression. Cereal grains are high in carbohydrates and ω -6 fatty acids (linoleic acid), but low in ω -3 fatty acids. Enrichment of food with ω -3 fatty acids is regarded as a way of increasing intake of these fatty acids and improving ratio ω -3/ ω -6 in order to reduce levels of risk of 'lifestyle' (Filipović, Pezo, Filipović, Brkljača, & Krulj, 2015; Monteiro et al., 2016; Rodríguez De Marco et al., 2018; Verardo et al., 2009). The fish powder is a by-product of fish processing and represents a cheap source of high quality nutrients (protein, EAA and fatty acid) that can be utilised in the human diet (Oliveira et al., 2015).

The quality of food product depends on the ability of dietary protein to supply the amino acid to meet the body requirement for performing metabolic function and it change with health and physiological conditions (Marinangeli & House, 2017). Bioavailability of indispensable amino acids (IAA) of food product not only depend on the protein and amino acid content, but also the quality of protein is also play an important role. Therefore, determination of amino acid profile of food product, together with its protein quality concerning its capacity to fulfil body's individual amino acid needs, is a key factor for satisfying consumer amino acid nutrition (Shaheen, Islam,

Munmun, Mohiduzzaman, & Longvah, 2016). So far, for the evaluation of protein quality of food product various methods tested including amino acid score, *in vitro* protein digestibility, protein efficiency ratio, net protein utilisation, protein efficiency ratio, *in vivo* protein digestibility, biological value, net protein retention, and protein digestibility corrected amino acid score (PDCAAS) (Coda et al., 2017; Nosworthy et al., 2017). These methods generally evaluate on the basis of digestibility, and bioavailability of amino acids and/or proteins. The PDCAAS has been used for more than 20 years to evaluate protein quality in human foods. However, the PDCAAS has been criticized for overestimating the number of amino acids absorbed, underestimating the higher nutritional value of animal proteins through truncation, and does not consider the effects of anti-nutritional factors and food processing (Schaafsma, 2005). To avoid errors, the Food and Agriculture Organization (FAO) now recommends an AA evaluation procedure called digestible indispensable amino acid score (DIAAS). DIAAS is the ratio of a milligram of digestible dietary IAA in one gram of the dietary protein to the milligram of the same dietary indispensable amino acid in one gram of the reference protein (FAO, 2013). DIAAS is preferred to, and regarded as more scientifically correct than, PDCAAS as it incorporates the latest scientific information regarding amino acid reference pattern, amino acid digestibility, and analytical methods to determine individual amino acids in foods. Instead of crude protein digestibility determined over the whole gastrointestinal tract, DIAAS considers individual amino acid digestibility determined at the end of the small intestine (or ileum) thus increasing the accuracy of the method. Unlike PDCAAS approach, DIAAS doesn't round down (truncate) scores to a maximum of 1.0 and thus recognizes the value of excess amino acids in higher quality proteins that can supplement comparatively lower quality proteins of cereals, for

example. Moreover, DIAAS rates protein sources considering the variation of amino acid requirement pattern of infants and children and employs three reference patterns: birth to 6 months, 6 months to 3 years, and 3–10 years (Leser, 2013). Therefore, the purpose of this study was to evaluate amino acid, fatty acid profile and to assess the quality of the pasta protein enriched with SFP by using digestible indispensable amino acid score (DIAAS).

8.2 Materials and methods

8.2.1 Raw materials

Described in section 3.1

8.2.2 Fish powder preparation

Described in section 3.1.1

8.2.3 Pasta production

Described in section 3.2.2

8.2.4 Chemical composition analysis of pasta

Described in section 3.4.1 to 3.4.4

8.2.5 Amino acid profile of pasta enriched with SFP

Described in section 3.4.7

8.2.6 Fatty acid profile of SFP added pasta

Described ion section 3.4.9

8.2.7 Calculation of digestible indispensable amino acid score (DIAAS)

Described in section 3.4.8

8.2.8 Statistical analysis

Described in section 3.4.22

8.3 Results and discussion

The protein content of the pasta products is most important and play a vital role in maintaining physiological function though their components amino acids. Table 8.1 shows the chemical composition of salmon powder enriched cooked pasta. The addition of salmon powder to semolina has been shown to affect the nutritional properties of the pasta. The incorporation of SFP resulted in level-dependent effects ($P < 0.05$) on the chemical composition of the pasta formulations.

Table 8.1 Chemical composition (g/100g) (on an as-is basis) of cooked pasta enriched with different salmon fish powder (SFP) levels.

Sample	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Moisture (g/100g)	Carbohydrat e (g/100g)
Semolina	12.7 ± 0.05	1.0 ± 0.01	1.1 ± 0.02	10 ± 0.10	72.8 ± 0.24
SFP	58.06 ± 0.13	38.55 ± 0.09	1.37 ± 0.06	2.15 ± 0.03	-
CP	12.88 ± 0.06 ^a	0.46 ± 0.02 ^e	0.39 ± 0.01 ^c	69.62 ± 0.92 ^a	16.63 ± 1.00 ^a
SFP5	15.41 ± 0.17 ^b	1.65 ± 0.15 ^d	0.48 ± 0.04 ^b	69.64 ± 0.71 ^a	13.11 ± 0.83 ^b
SFP10	18.10 ± 0.11 ^c	3.28 ± 0.28 ^c	0.52 ± 0.02 ^{ab}	66.09 ± 0.92 ^b	12.00 ± 0.99 ^b
SFP15	20.77 ± 0.09 ^d	5.61 ± 0.27 ^b	0.56 ± 0.01 ^a	66.69 ± 0.83 ^b	6.37 ± 0.91 ^c
SFP20	23.40 ± 0.13 ^e	7.20 ± 0.17 ^a	0.57 ± 0.03 ^a	68.15 ± 0.46 ^{ab}	0.67 ± 0.39 ^d

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation ($n=3$). Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The carbohydrate and moisture content decreased ($P < 0.05$) whereas the levels of protein, lipid, and ash increased ($P < 0.05$) with increasing levels of SFP. SFP had no carbohydrate compare to the semolina (72.8 %) and the protein (58.06 %), lipid (38.55 %), ash (1.37%) contents were significantly ($P < 0.05$) higher than semolina protein (12.70 %), lipid (1.65 %) and ash (1.1 %). Pasta enriched with SFP had higher protein content (15.41 to 23.40%) and lower fat content (1.65 to 7.20 %) as compared to egg

pasta protein (14.29 %) and fat (7.14 %) as reported by (Hager et al., 2013). A similar trend of an increase in protein, lipid, and ash contents has been reported for the pasta made with shrimp meat (Kadam & Prabhasankar, 2012), green mussel (Vijaykrishnaraj et al., 2014), tilapia (*Oreochromis niloticus*) flour (Monteiro et al., 2016), broad bean (Giménez et al., 2016), quinoa flour (Lorusso et al., 2017), legume flour (Bouasla et al., 2017) and carp fish powder (*Cyprinus carpio*) (El-Beltagi et al., 2017). The decrease in the moisture content may be attributed to a change in the protein-starch interaction when compared to the control pasta (Kadam & Prabhasankar, 2012). Table 8.2 illustrates the fatty acid profile of the pasta enriched with different levels of SFP. Regarding saturated fatty acids (SFA), pasta made with SFP exhibited greater ($P < 0.05$) amount of myristic acid (C14:0) and stearic acid (C18:0) whereas, lower ($P < 0.05$) palmitic acids (C16:0) level than the control pasta. All the formulations, showed significance difference ($P < 0.05$) to the control pasta in total SFA. SFP fortification increased the content of palmitoleic (C16:1), paullinic acid (C23:1) and vaccenic acid (C18:1) than the control pasta and resulting to increased ($P < 0.05$) of total monounsaturated fatty acid (MUFA) content in SFP added the pasta. The major PUFA present in control pasta was linoleic acid (18:2). ω -3 fatty acids were low in content with the ratio of ω -6: ω -3 being 19.17:1. This predominance of n-6 linoleic acid in cereal is a major contribution to the imbalanced ω -6: ω -3 ratio (Fradique et al., 2013). Increasing the percentage of SFP in the pasta formulation significantly enhanced EPA and DHA fatty acid content. In turn, the pasta prepared with SFP presented an important proportion of this LC-PUFAs in relation to other fatty acids.

Table 8.2 Fatty acid profile (g of individual fatty acids/100g of total fatty acids) of the cooked pasta enriched with different levels of SFP.

Fatty acid	CO	SFP 5	SFP 10	SFP 15	SFP 20
Saturated Fatty Acids (SFA)					
C14:0	nd	1.58 ± 0.003 ^d	1.77 ± 0.003 ^c	1.84 ± 0.006 ^b	1.92 ± 0.004 ^a
C16:0	21.63 ± 0.05 ^a	19.46 ± 0.04 ^b	19.02 ± 0.02 ^d	18.85 ± 0.03 ^e	19.15 ± 0.02 ^c
C18:0	1.45 ± 0.001 ^e	3.77 ± 0.001 ^d	4.29 ± 0.01 ^c	4.38 ± 0.01 ^b	4.53 ± 0.01 ^a
Monounsaturated Fatty Acids (MUFA)					
C16:1 n-7	nd	4.67 ± 0.007 ^d	5.34 ± 0.02 ^c	5.60 ± 0.01 ^b	5.83 ± 0.03 ^a
C18:1 n-9	12.45 ± 0.08 ^b	33.69 ± 0.05 ^a	37.92 ± 0.05 ^a	39.44 ± 0.06 ^a	40.60 ± 0.06 ^a
C20:1	nd	1.14 ± 0.01 ^d	1.31 ± 0.002 ^c	1.69 ± 0.002 ^b	1.71 ± 0.008 ^a
Polyunsaturated fatty acids (PUFA)					
C18:2 ω-6	60.39 ± 0.06 ^a	26.63 ± 0.03 ^b	20.17 ± 0.01 ^c	17.69 ± 0.04 ^d	15.83 ± 0.01 ^e
C18:3 ω-3	3.15 ± 0.02 ^a	2.03 ± 0.02 ^b	1.74 ± 0.007 ^c	1.65 ± 0.009 ^d	1.52 ± 0.002 ^e
C18:3 ω-6	nd	0.15 ± 0.008 ^c	0.18 ± 0.003 ^b	0.22 ± 0.002 ^a	0.22 ± 0.001 ^a
C20:2 ω-6	nd	0.27 ± 0.007 ^b	0.29 ± 0.001 ^a	0.30 ± 0.003 ^a	0.29 ± 0.001 ^a
C20:3 ω-6	nd	0.26 ± 0.004 ^b	0.27 ± 0.007 ^b	0.27 ± 0.001 ^c	0.26 ± 0.002 ^a
C20:4 ω-6	nd	0.40 ± 0.003 ^c	0.54 ± 0.004 ^a	0.55 ± 0.003 ^a	0.53 ± 0.002 ^b
C20:5 ω-3	nd	0.78 ± 0.002 ^c	0.93 ± 0.006 ^b	0.97 ± 0.003 ^a	0.92 ± 0.007 ^b
C22:5 ω-3	nd	0.39 ± 0.01 ^b	0.44 ± 0.004 ^a	0.45 ± 0.004 ^a	0.43 ± 0.003 ^a
C22:6 ω-3	nd	2.91 ± 0.005 ^d	3.11 ± 0.02 ^b	3.20 ± 0.01 ^a	3.05 ± 0.01 ^c
ΣSFA	23.08 ± 0.25 ^d	24.81 ± 0.10 ^c	25.08 ± 0.10 ^b	25.07 ± 0.09 ^b	25.60 ± 0.09 ^a
ΣMUFA	12.45 ± 0.09 ^e	39.50 ± 0.19 ^d	44.57 ± 0.14 ^c	46.73 ± 0.09 ^b	48.14 ± 0.10 ^a
ΣPUFA	63.55 ± 0.08 ^a	33.82 ± 0.01 ^b	27.66 ± 0.06 ^c	25.30 ± 0.08 ^d	23.04 ± 0.04 ^e
Σ ω-6	60.39 ± 0.06 ^a	27.71 ± 0.05 ^b	21.44 ± 0.03 ^c	18.98 ± 0.05 ^d	17.08 ± 0.02 ^e
Σ ω-3	3.15 ± 0.02 ^d	6.11 ± 0.04 ^b	6.21 ± 0.04 ^{ab}	6.27 ± 0.03 ^a	5.92 ± 0.02 ^c
EPA+DHA	-	3.69 ± 0.007 ^a	4.04 ± 0.02 ^c	4.18 ± 0.02 ^d	3.97 ± 0.02 ^b
ω6 : ω3	19.17:1	5:1	4:1	3:1	3:1

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CO: control sample. nd: not detected

Results in the table represent the mean of triplicate measurements. Mean ± SD (*n*=3).

Values within a row followed by the same superscript letter are not significantly different from each other (*P* > 0.05), according to Tukey's test.

Even in the pasta made with the minimum percentage of substitution (SFP5), it was possible to obtain an important reduction of the ratio ω-6: ω-3 (5:1) in comparison with the control pasta. From the human nutrition standpoint and prevention of cardiovascular disease, a diet with an ω-6/ω-3 ratio between 1 and 5 is recommended

by food agencies, scientific societies and national and international organisations (Agostoni, Bresson, & Chardigny, 2010). Research has indicated that inclusion of Japanese seaweed (*Undaria pinnatifida*), shrimp meat (*Penaeus monodon*) and carp fish powder (*Cyprinus carpio*) could raise this ratio (El-Beltagi et al., 2017; Prabhasankar et al., 2009; Ramya et al., 2015) respectively. The recommendations of EPA and DHA are 0.250–2 g/ day. The consumption of the pasta enriched with SFP provides 3.69 to 4.18 g of EPA and DHA, which represent almost double the minimum daily value recommended. Recent studies have shown that an intake of food rich in ω -3 LC-PUFA can have a positive effect on prevention of colon cancer and improved insulin sensitivity (Shahidi & Ambigaipalan, 2018). Polyunsaturated fatty acids are easily oxidised and both the drying and the cooking processes affect their stability, however, a significant amount of EPA and DHA remained stable in the pasta product. Future studies about the effects of the drying and cooking processes, of the pasta on EPA and DHA content are necessary. Amino acid composition of pasta plays a central role to the evaluation of protein quality. Also, protein quality of food depends on digestibility, chemical composition and presence of metabolic interfering substances that influence the utilisation of amino acids (Shaheen et al., 2016). Particularly, the indispensable amino acids (IAA) content in the pasta influences protein quality. Table 8.3 shows the amino acid profile of the pasta (mg AA/ g protein) enriched with salmon fish powder and control sample. The sum of IAA ranged from 330 mg in control sample and 371 mg to 452 mg/g protein in the pasta enriched with (5-20%) SFP. Lysine content was significantly highest in the pasta enriched with SFP (39 mg to 82 mg/g protein) than the control sample (19.24mg/g protein). In the present study, 20% SFP enriched pasta contained the highest (78 mg/g protein) isoleucine followed by 15% to 5% SFP (62 mg

to 37 mg/ g protein) as compared to control sample (34 mg/g protein). Valine content among the pasta enriched SFP samples (5-20 %) were significantly higher (39 mg to 42 mg/g protein) as compared to control sample (35 mg/g protein).

Table 8.3 Amino acid (AAs) composition (mg/g protein dry weight basis) of the cooked pasta enriched with salmon fish powder (SFP) and the control pasta.

Amino acid	CP	SFP5	SFP10	SFP15	SFP20
IAAs^a					
Phenylalanine	50.01 ± 0.38 ^a	48.70 ± 1.54 ^{ab}	43.20 ± 1.71 ^c	42.04 ± 0.88 ^c	45.24 ± 1.93 ^{bc}
Tyrosine	22.71 ± 0.47 ^c	25.08 ± 0.53 ^{bc}	25.83 ± 1.36 ^b	26.14 ± 0.42 ^b	29.15 ± 1.50 ^a
AAAs ^b	72.72 ± 0.61	73.78 ± 1.80	69.03 ± 2.39	68.18 ± 1.09	74.39 ± 2.68
Histidine	25.44 ± 0.48 ^a	26.51 ± 1.75 ^a	25.30 ± 3.05 ^a	28.68 ± 3.15 ^b	29.60 ± 0.79 ^b
Isoleucine	34.15 ± 0.47 ^d	36.73 ± 1.24 ^d	51.28 ± 4.33 ^c	62.36 ± 1.06 ^b	77.53 ± 2.55 ^a
Leucine	75.13 ± 1.17 ^b	81.06 ± 3.27 ^a	66.85 ± 0.99 ^c	61.40 ± 1.96 ^{cd}	62.74 ± 1.45 ^d
Lysine	19.42 ± 0.22 ^d	39.36 ± 1.22 ^c	73.66 ± 3.59 ^b	74.91 ± 1.39 ^b	82.50 ± 3.48 ^a
Methionine	11.18 ± 2.31 ^c	15.75 ± 0.16 ^b	17.80 ± 0.76 ^b	18.15 ± 1.49 ^b	22.57 ± 1.44 ^a
Cysteine	14.98 ± 0.17 ^a	12.18 ± 1.34 ^a	10.48 ± 3.45 ^a	11.42 ± 0.37 ^a	11.78 ± 0.72 ^a
SAAs ^c	26.16 ± 2.39	27.94 ± 0.83	28.28 ± 2.49	29.58 ± 1.67	34.35 ± 1.80
Threonine	27.41 ± 0.45 ^c	33.94 ± 0.90 ^b	33.21 ± 2.86 ^b	35.76 ± 0.75 ^b	40.32 ± 1.51 ^a
Tryptophan	13.40 ± 0.23 ^a	12.98 ± 0.39 ^a	12.41 ± 1.29 ^a	11.28 ± 1.96 ^{ab}	8.60 ± 0.36 ^b
Valine	35.82 ± 0.45 ^b	39.16 ± 1.17 ^{ab}	37.35 ± 2.66 ^b	38.54 ± 0.80 ^{ab}	42.41 ± 1.43 ^a
ΣIAAs	329.68 ± 6.36	371.46 ± 11.95	397.40 ± 23.32	410.79 ± 11.41	452.47 ± 16.49
DAAAs^d					
Arginine	38.92 ± 0.70 ^c	49.37 ± 1.77 ^b	50.61 ± 2.89 ^b	52.74 ± 1.40 ^b	60.67 ± 2.28 ^a
Alanine	29.58 ± 0.56 ^d	39.23 ± 1.37 ^c	41.88 ± 2.87 ^{bc}	45.72 ± 0.83 ^b	51.94 ± 1.98 ^a
Aspartic acid	33.53 ± 0.79 ^d	50.66 ± 1.79 ^c	54.57 ± 2.84 ^{bc}	60.12 ± 1.34 ^b	68.68 ± 2.63 ^a
Glutamic acid	544.57 ± 17.53 ^a	443.07 ± 40.62 ^b	252.47 ± 30.01 ^c	241.40 ± 2.90 ^c	243.98 ± 2.75 ^c
Glycine	38.17 ± 0.43 ^b	41.34 ± 1.44 ^{ab}	39.52 ± 2.08 ^b	40.60 ± 1.02 ^b	45.09 ± 2.04 ^a
Proline	127.46 ± 4.07 ^a	112.63 ± 4.34 ^a	76.51 ± 12.52 ^b	75.94 ± 2.17 ^b	72.92 ± 3.45 ^b
Serine	56.63 ± 1.20 ^a	55.36 ± 2.09 ^b	46.76 ± 5.82 ^{abc}	48.17 ± 0.99 ^{bc}	51.72 ± 1.93 ^c
ΣDAAAs	868.87 ± 24.26	791.66 ± 51.65	562.31 ± 10.18	564.60 ± 10.67	595.03 ± 17.09

The italic values indicate the sums from two previous. For example, AAA (aromatic amino acids) is the sum of Phenylalanine and Tyrosine. Bold value indicate the grand totals for Indispensable amino acids (ΣIAAs) and Dispensable amino acids (ΣDAAAs)

^a Indispensable Amino Acids ;^b Aromatic Amino Acids (Phenylalanine + Tyrosine); ^c Sulphur Amino Acids (Cysteine+ Methionine); ^d Dispensable Amino Acids.

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation ($n=3$). Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

Pasta with 20% SFP was found to contain the highest amount of threonine (40 mg/g protein) followed by 15% (36 mg/g protein), 10% (33 mg/g protein), 5% (34 mg/g protein) and lowest was in control sample (24 mg/g protein). Histidine was found to be significantly ($P < 0.05$) rich in the pasta enriched with SFP (5-20%) that has different function including protein interaction and tissue repair and growth (Usydus, Szlinder-Richert, & Adamczyk, 2009). Similar findings were reported by (Ramya et al., 2015), (Prabhasankar et al., 2009) and (El-Beltagi et al., 2017) in the pasta elaborated with shrimp meat and Japanese seaweed and pizza fortified with carp fish powder (*Cyprinus carpio*). Of all the individual IAA, lysine was the most available amino acid in all the pasta enriched sample except the control pasta. The control pasta sample was deficient in tyrosine. Relative deficiencies of phenylalanine, leucine and tryptophan were observed in the enriched pasta sample and those of lysine, SAAs, histidine, isoleucine, threonine and valine in the control pasta can be due to the composition of storage proteins (actin and myosin in the pasta enriched with SFP) and prolamins in the control pasta. The dispensable amino acids (DAAs) termed functional amino acids, include arginine, alanine, aspartic acid, glutamic acid, glycine, proline and serine play important function in intestinal integrity (Dai, 2011), metabolism of nutrient (Lager & Powell, 2012), gene expression and antioxidative responses (Wu et al., 2013) and immune responses (Wu, 2013). As shown in Table 8.3 glutamic acid was the predominant amino acid amongst the DAAs ranging from 545 mg/g protein in the control pasta and 443 to 243 mg/g protein in the enriched pasta (5-20%) respectively. Arginine (49 to 61 mg/g protein), alanine (39 to 52 mg/g protein) and glycine (41 to 45 mg/g protein) content of the

enriched pasta (5-20%) were found to be higher than the control pasta. Aspartic acid is a precursor of other amino acids, and was found to be highest (50 to 69 mg/protein) in the pasta with SFP than control pasta (33 mg/g protein).

Table 8.4 Adult daily recommended allowances of indispensable amino acids (IAA) and their composition (mg/g of proteins) in control and the pasta enriched with (SFP).

EAA	Adult daily requirement ^d		CP	SFP 5	SFP 10	SFP 15	SFP 20
	mg/kg/day	mg/g protein					
AAA ^a	25	38	73	74	69	68	74
Histidine	10	15	25	27	25	29	30
Isoleucine	20	30	34	37	51	62	78
SAA ^b	15	22	26	28	28	30	34
Valine	26	39	36	39	37	39	42
Leucine	39	59	75	81	67	61	63
Lysine	30	45	19	39	74	75	83
Threonine	15	23	27	34	33	36	40
Tryptophan	4	6	13	13	12	11	9
ΣIAA (%TAA ^c)	184	277	328	372	396	411	453
			(27.5)	(31.98)	(41.26)	(42.13)	(43.24)

Aromatic Amino Acids (Phenylalanine +Tyrosine); ^b Sulphur Amino Acids (Cysteine+ Methionine)

^c Total amino acids; ^d FAO/WHO/UNU (2007) adult indispensable amino acid requirements pattern (FAO, 2013); SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta.

Table 8.4 depicts a comparative overview of the amounts of indispensable amino acid (IAA) in the control pasta and the pasta enriched with SFP with the FAO/WHO/UNU adult IAA requirements (WHO/FAO/UNU, 2007). As suggested by FAO/WHO/UNU for adult human's requirement, the pasta enriched with SFP (5-20%) were found to have higher IAA levels than the control pasta. The total IAA contents (mg IAA/g protein) of all the pasta samples exceeded the recommended daily allowance (277 mg IAA/g protein) and contributed on average 41% to total amino acids (TAA) contents (Table 8.3). The control pasta contained a relatively low proportion of IAA to TAA (27.5%) among the

pasta enriched with 5-20% SFP. The pasta enriched with 20% SFP had relatively higher amount of IAA (43%) followed by 42%, 41%, and 32% to total amino acid (TAA) for the pasta with 15, 10 & 5% SFP respectively. A higher proportion of IAA in the pasta enriched with SFP in the present study together with greater satiety effect compared to beef and chicken protein (Borzoei, Neovius, Barkeling, Teixeira-Pinto, & Rossner, 2006) suggest the pasta enriched with SFP to be considered as for importance dietary protein source. In 2013, the FAO recommended a new method of assessing the protein quality of food products, digestible indispensable amino acid score (DIAAS).

Table 8.5 Digestible IAA content (mg/g protein) for each IAA of the cooked control pasta and the pasta enriched with SFP.

Amino acid	CP	SFP5	SFP10	SFP15	SFP20
Lysine	17.29	36.12	67.77	68.92	75.90
Threonine	24.40	31.90	31.21	33.61	37.90
Tryptophan	12.19	11.68	11.17	10.15	7.74
Methionine	10.40	14.17	16.02	16.34	20.31
Cysteine	13.93	10.96	9.43	10.28	10.60
Leucine	70.62	72.95	60.16	55.32	56.47
Isoleucine	31.41	33.79	47.18	55.37	71.32
Phenylalanine	47.51	40.42	35.85	34.89	37.54
Tyrosine	21.35	22.56	23.24	23.53	26.23
Valine	32.23	34.85	33.25	34.30	37.75
Histidine	24.16	22.26	21.25	24.09	24.86

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta. Mean \pm standard deviation ($n=3$).

DIAAS is based on the lowest amount of the digestible indispensable amino acid per unit of the dietary protein (Leser, 2013). It considers the digestibility of individual amino acids estimated at the end of the small intestine (or ileum) and recognises the value of excess amino acids in higher quality proteins that can supplement comparatively lower quality proteins as those in cereal. Also, DIAAS rates protein sources according to the

variation of amino acid requirement pattern of infants and children (FAO, 2013). Table 8.5 illustrates digestible IAA content for each IAA in 1 g protein of the pasta samples. It was calculated by multiplying the IAA content by true ileal digestibility coefficient of the corresponding IAA. Pasta enriched with SFP showed increase in the digestible IAA content as compared to the control pasta. Table 8.6 and 8.7 describe the DIAAS values for the control pasta and the pasta enriched with different levels (5-20%) of SFP.

Table 8.6 Dietary Indispensable amino acids (IAA) reference ratio for minimal IAA and digestible indispensable amino acid score (DIAAS) of the control pasta and the pasta enriched with (SFP).

	Amino acid scoring pattern :young child ^a				
	CP	SFP5	SFP10	SFP15	SFP20
DIAA reference ratio					
AAA	1.33	1.21	1.14	1.12	1.23
Histidine	1.21	1.11	1.06	1.20	1.24
Isoleucine	0.98	1.06	1.47	1.79	2.29
Leucine	1.07	1.11	0.91	0.84	0.86
Lysine	0.30	0.64	1.19	1.20	1.33
SAA	0.90	0.93	0.94	0.99	1.15
Threonine	0.79	1.03	1.00	1.08	1.22
Tryptophan	1.43	1.37	1.31	1.19	0.91
Valine	0.75	0.81	0.77	0.80	0.87
DIAAS (% (IAA))	30 (Lys)	64 (Lys)	77 (Valine)	80 (Valine)	86 (Leu)

AAA: aromatic amino acids (Phenylalanine +Tyrosine); DIAA: digestible indispensable amino acids; DIAAS: digestible indispensable amino acid score; SAA: sulphur amino acids (Cysteine+ Methionine); ^a The IAA reference pattern are expressed as mg amino acid/g protein: Histidine, 20; Isoleucine, 32; Leucine, 66; Lysine, 57; SAA, 27; AAA, 52; Threonine, 31; Tryptophan, 8.5; Valine, 43 (FAO, 2013).

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta. Mean \pm standard deviation ($n=3$).

Table 8.7 Dietary Indispensable amino acids (IAA) reference ratio for minimal IAA and digestible indispensable amino acid score (DIAAS) of the control pasta and the pasta enriched with (SFP).

	Amino acid scoring pattern: older child, adolescent and adult ^b				
	CP	SFP5	SFP10	SFP15	SFP20
DIAA reference ratio					
AAA	1.68	1.53	1.44	1.42	1.55
Histidine	1.51	1.39	1.33	1.51	1.55
Isoleucine	1.05	1.13	1.57	1.91	2.37
Leucine	1.15	1.20	0.98	0.91	0.93
Lysine	0.36	0.75	1.41	1.43	1.58
SAA	1.05	1.09	1.11	1.15	1.34
Threonine	0.97	1.27	1.25	1.34	1.52
Tryptophan	1.84	1.77	1.69	1.54	1.17
Valine	0.80	0.87	0.83	0.86	0.94
DIAAS (%) (IAA))	36 (Lys)	75 (Lys)	83 (Val)	86 (Val)	93 (Leu)

AAA: aromatic amino acids (Phenylalanine +Tyrosine); DIAA: digestible indispensable amino acids; DIAAS: digestible indispensable amino acid score; SAA: sulphur amino acids (Cysteine+ Methionine)' ^b The IAA reference pattern are expressed as mg amino acid/g protein: Histidine, 16;Isoleucine,30; Leucine, 61; Lysine, 48;SAA,23; AAA,41;Threonine,25; Tryptophan,6.6; Valine, 40 (FAO, 2013).

SFP5, SFP10, SFP15, and SFP20: pasta prepared with 5, 10, 15, and 20 g of salmon fish powder /100 g of semolina flour. CP: control pasta. Mean \pm standard deviation ($n=3$).

With reference to DIAAs reference pattern of a young child, the control pasta value is 30% (lysine), compared to 64% (lysine) to 86% (leucine) for the pasta made with 5, 10, 15 and 20% SFP respectively. When calculating the DIAAS using the standard reference pattern of older child, adolescent, and adult, the pasta enriched with 20% SFP was ranked first (DIAAS: 93% (Leucine) followed by 15% SFP pasta (DIAAS: 86% (Valine), 10% SFP pasta (DIAAS: 83% (Valine) and 5% SFP pasta (DIAAS: 75 % (Lys). The DIAAs cut-off values, grade protein quality as 'Excellent', if DIAAS greater than 100; 'Good', if DIAAS between 75 and 99; and 'Low' if DIAAS below 75. Based on these cut-off values, in this study, the pasta made with semolina (control pasta) was of "Low" quality (DIAAS < 75%) and this is in agreement with previous (Prabhasankar et al., 2009; Ramya et al., 2015).

This 'Low' quality can be attributed to the deficiencies of lysine in the control pasta as revealed by their amino acids composition. Pasta enriched with SFP showed 'Good' quality (DIAAS > 100%) protein source. This indicates that these salmon fish powder contain a sufficient amount of indispensable amino acids that can be used to prepare the pasta like product and enhance the protein quality.

8.4 Conclusion

To summarize, in the present study, the pasta enriched with SFP (5-20%) provided an enhanced nutritional status via increased levels of protein, indispensable amino acids (IAA) such as lysine and fatty acids such as EPA and DHA. The IAA composition of the enriched pasta with SFP is more beneficial to compensate for the lysine deficiency of semolina made the pasta. The incorporation of SFP (5-20 %) in the pasta formulation allowed to obtain lower n6:n3 ratio (5:1 to 3:1), respectively) as compared with the control pasta. Furthermore, on the basis of DIAAS, the pasta made SFP could serve as the good sources of protein than semolina pasta. For children (6 months to 3 years) and children older than 3 years, the most limiting AA in the control pasta was Lysine and the DIAAS was 36 whereas in the pasta elaborated with SFP showed DIAAS above 75. Regardless of the scoring pattern used, the DIAAS values of the control pasta protein in the present study did not satisfy the minimum value that is recommended to make claims for protein quality of foods (FAO, 2013) indicating that the control pasta needs to be supplemented with proteins rich ingredient with greater concentrations of Lysine such as salmon powder, to fulfil the AA requirements of consumer.

Chapter 9

Physicochemical and nutritional characteristics of wheat bread enriched with cod fish (*Pseudophycis bachus*) powder

This chapter is submitted for publication for “**Journal of Food Biochemistry**”

Ajay S. Desai., Margaret A. Brennan., & Charles S. Brennan. Physicochemical and nutritional characteristics of wheat bread enriched with cod fish (*Pseudophycis bachus*) powder.

Abstract

The aim of this study was to evaluate physical (moisture, volume), technological (texture and colour) nutritional (amino acid content, starch and protein digestibility) and nutraceutical (total phenolic content (TPC) and antioxidant capacity) properties of bread fortified with different levels (5 %, 10 % and 15 %) of cod fish (*Pseudophycis bachus*) powder (CFP) and compared with a control bread. Bread fortification showed a significant effect on bread properties depending on fortification level. There was significant ($P < 0.05$) effect of CFP on bread specific volume, crumb colour, and textural properties. The nutritional quality of the bread was analysed using an *in vitro* glycaemic response and protein digestibility digestion method. The results illustrate that the incorporation CFP into wheat flour decreased the potential glycaemic response of bread. However, this study also shows that addition of CFP increased ($P < 0.05$) protein and phenolic content, antioxidant capacity and protein digestibility of breads with the corrected amino acid score (PDCAAS) of fortified breads being higher ($P < 0.05$) than the control bread.

9.1 Introduction

Bread is a staple food consumed in the daily diet of most of the populations with production of 143 metric tonnes in 2016 (Bread Forecast, 2025). Traditionally bread is produced with wheat flour however the protein in bread is often regarded as low quality as it is usually deficient in one or more essential amino acids (EAA) such as lysine and methionine. The amino acid concentration in the diet is an important factor in the nutritional value of a food protein. Consumer attention has become focused on healthier diets and the prevention of diseases such as cardiovascular disease, type-2 diabetes, colon cancer and obesity (Coelho & Salas-Mellado, 2015), such that there has been an increased demand for enrichment of food by the food industry. Consumers are desirous of improved physical (volume, colour and texture), nutritional (amino acid content, protein digestibility, starch digestibility) and antioxidant properties (Liu, Brennan, Serventi, & Brennan, 2017a). Enrichment of food is an effective way to achieve products that are characterized by an increased content of health promoting components. According to the Food and Drug Administration (FDA) and the World Health Organization (WHO) bread is considered as an ideal vehicle for the addition of nutrients to diet, since it can be fortified with protein rich ingredients (Dziki, Rozylo, Gawlik-Dziki, & Swieca, 2014). Starch and protein are the major components of wheat bread. Addition of protein rich ingredients affects the physical and nutritional properties of wheat bread by disturbing the gluten network. Over the past few decades, bread has been prepared incorporating different ingredients including, onion skin (Gawlik-Dziki et al., 2013), amaranth, buckwheat, chickpea and quinoa flours (Burešová et al., 2017). Wheat flour contains less than 1% resistant starch which makes wheat bread a high glycaemic index food. The consumption of rapidly digestible starchy food

can lead to the development of metabolic syndrome. Consumption of foods with a low glycaemic value could manipulate the effects of diabetes, cardiovascular and neurodegeneration. Low glycaemic index foods can be achieved by the utilizing protein rich and fibre rich ingredients in combination with cereal grains in products such as bread. In recent years, bread products have been fortified with high protein sources such as carob, faba bean, fish (Turfani, Narducci, Durazzo, Galli, & Carcea, 2017), (Coda et al., 2017) and cobia (Fagundes et al., 2018). Phenolic compounds exhibit biological properties, such as antioxidant activity. Foods rich in polyphenols have the potential to protect against various diseases associated with oxidative damage, such as cardiovascular, cancer and neurological disease (Sęczyk et al., 2016a). Proteins and amino acid derived from fish are considered nutritionally superior to that of plant origin. Fish is not only excellent source of high value protein but also important source of EAA (lysine, methionine and threonine) and ω -3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which is related to health benefits, such as the prevention of cardiovascular disease, hypertension, cancer and diabetes as well as containing micronutrients such as vitamins (A, D, B6 and B12) and minerals (iron, zinc, iodine, selenium, potassium and sodium) (Dalton et al., 2009). The American heart association (AHA) recommends a minimum consumption of two fish serving per week (200mg/day of long chain ω -3 polyunsaturated fatty acid (PUFA)) to achieve a cardio protective effect. To fulfil these requirements bread could be fortified with fish powder. Fish powder is protein rich which contributes to a low glycaemic index and gives the potential to have beneficial health effects such as manipulation of obesity, hypertension and cardiovascular disease (Kadam & Prabhasankar, 2010). However, the physical and nutritional properties of bread enriched with partial replacement of wheat flour by cod

powder (*Pseudophycis bachus*) are still unknown. Therefore, this study was conducted to evaluate the effects of addition of different levels of CFP on the bread characteristics including *in vitro* starch and protein digestibility and antioxidant activity.

9.2 Materials and methods

9.2.1 Raw materials

Described in section 3.1

9.2.2 Fish powder preparation

Described in section 3.1.1

9.2.3 Preparation of bread

Described in section 3.2.3

9.2.4 Proximate chemical composition analysis of bread

Described in section 3.4.1 to 3.4.4

9.2.5 Volume, density, moisture and texture properties of bread

Described in section 3.4.11.6

9.2.6 Colour measurements

Described in section 3.4.13.4

9.2.7 Gastric digestibility using *In vitro* starch digestion process

Described in section 3.4.6

9.2.8 Amino acid profile and scoring

Described in section 3.4.7

9.2.9 *In vitro* protein digestibility and protein digestibility corrected amino acid score (PDCAAS)

Described in section 3.4.5

9.2.10 Total phenolic content and antioxidant activity of bread

Described in section 3.4.12.1 and 3.4.12.2

9.3 Results and discussion

9.3.1 Chemical composition, energy content and specific volume of bread

The chemical composition and energy value of the raw material and breads enriched with different levels of CFP are presented in Table 9.1

Table 9.1 Bread formulations, proximate compositions (on an as-is basis) and physical properties of bread. A. Ingredients used in cod powder (CP) enriched bread. B. Proximate composition of bread elaborated with CP. C. Physical properties of bread made with CP.

A						
Sample code						
Ingredients (g)	CB	CB5	CB10	CB15		
Wheat flour	150	142.5	135	127.5		
Cod powder	-	7.5	15	22		
salt	2.25	2.25	2.25	2.25		
Sugar	9	9	9	9		
Yeast	2.25	2.25	2.25	2.25		
Butter	7.5	7.5	7.5	7.5		
Water	90	90	90	90		
B						
Sample code	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	Energy (Kcal/100g)
CB	13.91 ± 0.19 ^d	3.86 ± 0.02 ^b	2.13 ± 0.02 ^d	34.38 ± 0.55 ^a	80.10 ± 0.18 ^a	410.8 ± 0.18 ^a
CB5	17.46 ± 0.16 ^c	3.55 ± 0.03 ^c	2.51 ± 0.04 ^c	31.65 ± 0.42 ^b	76.49 ± 0.17 ^b	407.71 ± 0.30 ^{bc}
CB10	21.23 ± 0.09 ^b	3.61 ± 0.05 ^c	2.70 ± 0.02 ^b	33.09 ± 0.47 ^{ab}	72.46 ± 0.07 ^c	407.25 ± 0.28 ^c
CB15	23.56 ± 0.12 ^a	3.96 ± 0.01 ^a	2.87 ± 0.06 ^a	31.94 ± 0.73 ^b	69.61 ± 0.14 ^d	408.30 ± 0.26 ^b

C			
Sample code	Width/ height ratio (mm)	Volume (mL)	Specific volume (mL/g)
CB	1.80 ± 0.12^a	111 ± 1.00^a	2.47 ± 0.00^c
CB5	1.84 ± 0.38^b	92 ± 2.00^b	2.05 ± 0.01^b
CB10	1.86 ± 0.15^b	90 ± 0.58^{bc}	2.04 ± 0.00^b
CB15	2.01 ± 0.10^c	89 ± 2.31^c	2.02 ± 0.02^b

CB5, CB10 and CB15: bread prepared with 5, 10 and 15g of cod fish powder /100 g of wheat flour. CB: control bread.

Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The incorporation of 5-10 % CFP to the formulations increased ($P < 0.05$) the levels of protein, fat, ash content and decreased ($P < 0.05$) the moisture and carbohydrate content compared to those of the control bread. The protein content of bread enriched with CFP ranged from 17.43 to 23.56 % compared to 13.91 % in the control bread. The lipid content of CFP enriched breads varied from 3.55 to 3.96 %. The 15 % CFP supplemented bread exhibited significantly increased lipid content compared to the control bread. The ash content of CFP breads ranged from 2.51 to 2.87 % which was different ($P < 0.05$) to the control bread. Among the CFP enriched breads significant effects were observed. The moisture content of the control bread was 34.38 % which was significantly higher than bread supplemented with CFP. Lower moisture content in CFP enriched bread may be due to the protein-polysaccharide interaction and low water retention capacity compared to the control bread. The energy value of CFP enriched breads was lower than the control bread. Lipid and carbohydrate contents of CFP bread showed that there was decrease in the contents. Similar results were obtained by Fagundes, et al., (2018) for bread fortified with cobia (*Rachycentron canadum*) powder.

The incorporation of CFP decreased loaf volume from 111 to 89 mL and specific volume from 2.47 to 2.02 mL/g (Table 9.1c). The width/height ratio of bread enriched with CFP increased in comparison with control bread. It has been proved that gluten is the major visco-elastic protein which helps to shape and raise the bread (Turfani et al., 2017), the decrease in volume could be due to the reduction in gluten content as a result of CFP supplementation in bread. Such a dilution of gluten and the interaction of CFP protein (non-gluten protein) in the gluten matrix during the processing of the breads may reduce the ability of CFP breads to extend and hold the carbon dioxide produced during fermentation. Our finding is in agreement with (Turfani et al., 2017) who prepared bread enriched with lentil flour and found that loaf volume reduced significantly.

9.3.2 Texture and colour measurement of bread

The texture and colour of bread are considered to be very important factors in product commercialisation. From the consumer point of view, the development of texture parameters is a critical point to ensure the acceptance of products. The textural properties of bread are mainly controlled by a gluten network, which is a structural network of starches, protein additions, and other ingredients. The bread enriched with different levels of CFP showed significantly ($P < 0.05$) higher hardness values (1592 to 2112 g) compared to control bread (1082 g) Table 9.2A. In case of gumminess and chewiness, a significant ($P < 0.05$) increase were observed in CFP (5-15%) enriched breads with regard to the control bread. However, springiness, cohesiveness, and resilience were decreased ($P < 0.05$) of bread supplemented with CFP compared to control bread Table 9.2A. The hardness of bread is related to the peak force required to compress the sample, while chewiness represents a quantitative estimation of energy

needed to disintegrate bread structure (Liu et al., 2017a). Springiness represents the capacity of sample to spring back after a deformation due to the compression.

Table 9.2 Technological characteristic of bread enriched with different levels of cod powder (CB). A. Texture profile analysis. B. Colour characteristics of crust and crumb.

A				
Parameter	CB	CB5	CB10	CB15
Hardness (g)	1082.77 ± 0.20 ^c	1592.47 ± 93.09 ^b	1941.05 ± 201.68 ^a	2112.44 ± 61.01 ^a
Springiness (mm)	0.935 ± 0.003 ^a	0.912 ± 0.013 ^b	0.871 ± 0.009 ^c	0.867 ± 0.007 ^c
Gumminess (g)	788.99 ± 5.01 ^c	889.87 ± 15.88 ^{bc}	962.11 ± 86.19 ^b	1198.07 ± 67.38 ^a
Chewiness (g)	737.25 ± 10.79 ^c	811.56 ± 33.26 ^b	837.99 ± 82.60 ^b	1038.72 ± 30.62 ^a
Cohesiveness	0.73 ± 0.01 ^b	0.51 ± 0.01 ^a	0.56 ± 0.02 ^d	0.61 ± 0.02 ^c
Resilience	0.35 ± 0.01 ^a	0.28 ± 0.01 ^b	0.24 ± 0.02 ^c	0.25 ± 0.01 ^{bc}
B				
Sample code	Crust colour			
	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	ΔE
CB	91.45 ± 0.32 ^a	9.97 ± 0.27 ^a	36.65 ± 0.07 ^a	-
CB5	89.79 ± 0.21 ^b	5.20 ± 0.21 ^b	35.62 ± 0.09 ^a	5.15 ± 0.45 ^a
CB10	84.68 ± 0.90 ^c	3.30 ± 0.11 ^d	31.91 ± 0.91 ^b	10.66 ± 0.98 ^b
CB15	82.28 ± 0.33 ^d	2.59 ± 0.17 ^c	28.61 ± 0.41 ^c	14.26 ± 0.25 ^c
Sample code	Crumb colour			
	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	ΔE
CB	95.31 ± 0.12 ^b	12.23 ± 0.02 ^c	30.92 ± 0.08 ^b	-
CB5	96.85 ± 0.12 ^a	13.06 ± 0.24 ^a	32.39 ± 0.56 ^a	2.32 ± 0.40 ^a
CB10	96.55 ± 0.28 ^a	12.92 ± 0.05 ^{ab}	32.35 ± 0.26 ^a	2.05 ± 0.08 ^a
CB15	96.89 ± 0.15 ^a	12.72 ± 0.05 ^b	31.85 ± 0.03 ^a	1.91 ± 0.26 ^a

CB5, CB10 and CB15: bread prepared with 5, 10 and 15g of cod fish powder /100 g of wheat flour. CB: control bread.

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

Enrichment with protein rich CFP influenced the bread's hardness. This might be due to the thickening of gas cell walls within the bread crumb due to the high water absorption characteristics of CFP protein. Additionally, the increased protein and

decreased lipid content in bread enriched with CFP might interfere with gas retention and create more unstable gas cells resulting in a compact structure giving it a chewy crumb making it harder. Phongthai et al. (2016) and Fagundes et al. (2018) reported that 4% inclusion of egg albumin and 12% cobia fish protein resulted in a significant ($P < 0.05$) increase in bread firmness.



Figure 9.1 Bread enriched with cod powder

The lightness (L^*), redness (a^*), and yellowness (b^*) values of crust and crumb of bread enriched with CFP are shown in Table 9.2B and Figure 9.1. As the levels of CFP increased, the L^* , a^* , b^* of bread crust decreased. Bread fortified with 20% CFP provided the lowest L^* , a^* , b^* . For the bread crumb, the L^* , a^* , b^* values increased as the level of CFP increased. Bread colour is a result of complex reactions mainly dependant on the physicochemical characteristics of dough (water, starch and lysine content) and temperature used during baking process. The addition of CFP altered the crumb colour of CFP enriched bread to be more yellowish (b^*). The darkening of CFP breads might have been attributed to an increased Maillard reaction taking place during baking

process due to the higher lysine content. In the Maillard reaction reducing carbohydrates react with free amino acid side chain of protein mainly lysine and lead to amino acid-sugar reaction products (Turfani et al., 2017). The results obtained by Sanz-Penella et al. (2013) are similar to the observation of low and high L^* and b^* values in bread crust and crumb respectively. They reported that lightness and yellowness value of bread crust and crumb increased as the level of amaranth flour (10-40%) powder increased in bread. Also, Turfani et al. (2017) demonstrated that incorporation of 6, 12 and 24% lentil flour in bread had significantly different yellowness and lightness characteristics to those of control. The ΔE values were determined to evaluate the colour differences between the control and the CFP enriched formulations. The ΔE values of bread crust increased ($P < 0.05$) with increasing levels of CFP but no significant differences were observed in the bread crumb. In addition, bread crust exhibited higher ΔE compared to the bread crumb, indicative of the colour compounds released during baking process. The ΔE values were more than 3.0 for bread crust, and below 3.0 for bread crumb. According to handbook of colour science, these values fall in the “appreciable, detectable by ordinary people” and “noticeable, detectable by trained people” respectively. Reshmi et al. (2017) reported that bread enriched with dried pomelo fruit segments showed higher ΔE value in bread crust with increasing level 2-7.5%.

9.3.3 Protein quality of bread

9.3.3.1 Amino acid profile and amino acid scoring of bread samples

The EAA contents of bread enriched with CFP and control are presented in Table 9.3. CFP inclusion increased ($P < 0.05$) the levels of EAA such as lysine, leucine, isoleucine, methionine, threonine, and valine. Amongst the EAA, lysine, methionine,

and threonine increased ($P < 0.05$) in a level dependent manner on CFP addition. While, the levels of tyrosine, isoleucine, and leucine and valine were increased higher ($P < 0.05$) by 10% and 15% CFP inclusions. The contents of phenylalanine and cysteine decreased in fortified bread with CFP compared to control.

Table 9.3 Amino acid (AAs) composition (mg/g protein dry weight basis) of bread enriched with different levels of cod fish powder and control.

A				
Amino acid	CB	CB5	CB10	CB15
Phenylalanine	42.24 ± 0.19 ^a	40.37 ± 0.49 ^{ab}	37.92 ± 0.22 ^b	39.11 ± 3.06 ^{ab}
Tyrosine	18.66 ± 2.08 ^b	21.55 ± 5.49 ^{ab}	22.07 ± 2.02 ^{ab}	27.12 ± 0.21 ^a
Histidine	20.75 ± 1.33 ^a	20.32 ± 0.58 ^a	19.18 ± 0.36 ^a	20.44 ± 1.59 ^a
Isoleucine	30.31 ± 0.81 ^b	33.14 ± 0.24 ^{ab}	33.53 ± 0.09 ^{ab}	37.11 ± 1.33 ^a
Leucine	62.18 ± 0.57 ^b	66.80 ± 0.88 ^{ab}	67.04 ± 0.05 ^{ab}	71.88 ± 5.69 ^a
Lysine	9.69 ± 1.14 ^d	25.46 ± 0.15 ^c	29.01 ± 0.37 ^b	36.32 ± 2.42 ^a
Methionine	10.88 ± 0.65 ^c	15.20 ± 0.90 ^b	17.04 ± 0.60 ^b	20.04 ± 0.84 ^a
Cysteine	11.69 ± 0.98 ^a	9.52 ± 1.59 ^{ab}	8.54 ± 0.57 ^b	8.53 ± 0.32 ^b
Threonine	23.22 ± 0.22 ^c	28.43 ± 0.09 ^b	29.82 ± 1.10 ^{ab}	32.14 ± 2.59 ^a
Valine	32.17 ± 0.90 ^b	35.22 ± 0.16 ^{ab}	35.18 ± 0.10 ^{ab}	38.72 ± 3.36 ^a
ΣEAAs	261.79 ± 9.23 ^a	296.01 ± 8.69 ^b	299.33 ± 7.89 ^b	331.41 ± 9.66 ^c
B. Amino acid score ^a				
Histidine	1.15 ± 0.08 ^a	1.12 ± 0.04 ^a	1.06 ± 0.02 ^a	1.13 ± 0.09 ^a
Isoleucine	0.98 ± 0.03 ^b	1.07 ± 0.01 ^{ab}	1.08 ± 0.01 ^{ab}	1.19 ± 0.10 ^a
Leucine	0.99 ± 0.01 ^b	1.06 ± 0.02 ^{ab}	1.06 ± 0.00 ^{ab}	1.14 ± 0.09 ^a
Lysine	0.19 ± 0.02 ^c	0.49 ± 0.01 ^b	0.56 ± 0.01 ^b	0.69 ± 0.05 ^a
Phenylalanine + Tyrosine	1.32 ± 0.05 ^a	1.34 ± 0.10 ^a	1.30 ± 0.05 ^a	1.44 ± 0.06 ^a
Methionine + Cysteine	0.87 ± 0.06 ^b	0.95 ± 0.10 ^{ab}	0.98 ± 0.05 ^{ab}	1.10 ± 0.04 ^a
Threonine	0.86 ± 0.02 ^b	1.05 ± 0.01 ^a	1.10 ± 0.04 ^a	1.19 ± 0.10 ^a
Valine	0.77 ± 0.03 ^b	0.84 ± 0.00 ^{ab}	0.84 ± 0.01 ^{ab}	0.92 ± 0.08 ^b
C. <i>In vitro</i> protein digestibility (%) & PDCAAS				
<i>In vitro</i> protein digestibility (%)	79.96 ± 0.65 ^b	81.40 ± 0.10 ^a	82.19 ± 0.18 ^a	81.53 ± 0.20 ^a
PDCAAS ^b	0.15 ± 0.65 ^a	0.39 ± 0.95 ^b	0.46 ± 1.15 ^c	0.56 ± 1.05 ^d

Bold value indicate the grand totals for Essential amino acids (**ΣEAAs**) and nonessential amino acids (**ΣNEAAs**). CB5, CB10 and CB15: bread prepared with 5, 10 and 15 g of cod fish powder /100 g of semolina flour. CB: control bread.

^a Based on standard FAO/WHO/UNU (2007) 1-2 year old reference pattern (mg/g protein): Histidine-18; Lysine-52; Isoleucine-31; Leucine-63; Methionine + Cysteine-26; Phenylalanine + Tyrosine-46; Threonine-27; Valine-42.

^b Protein digestibility corrected amino acid score (PDCAAS): AAS (lowest score of an individual amino acid) \times *In vitro* protein digestibility of pasta sample.

Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

No difference was observed in histidine content. Bread prepared with different levels of CFP (CFP 15% > CFP10 > CFP5%) exhibited greater ($P < 0.05$) total EAA contents than control. Bread products manufactured with wheat flour contain a limited amount of EAA representing potential target for the incorporation of protein sources such as fish products (Kadam & Prabhasankar, 2010). (Coda et al., 2017) reported a similar pattern for total EAA levels in bread enriched with faba bean. The EAA scores of the bread enriched with CFP and control are presented in Table 9.3.

The breads with CFP, had a significantly higher lysine, isoleucine, leucine, methionine + cysteine, threonine and valine score but lower scores for histidine. There was no significance in phenylalanine + tyrosine scores compared to the control sample. The amounts of all the EAA analysed, isoleucine, leucine, lysine, methionine cysteine, threonine and valine in bread fortified with CFP were higher (amino acid scores) compared to the standards for amino acids of ideal reference protein, appropriate for children aged 1–2 (which also covers the range appropriate for human adults) (FAO/WHO, 2007). Based on the EAA scores (Table 3), the limiting amino acid is lysine for all bread with CFP and control bread, therefore the scores for lysine were used to calculate the PDCAAS of the breads. A higher level of CFP addition would, therefore, be required to further improve the lysine score of the bread.

9.3.3.2 *In vitro* protein digestibility (IVPD) and protein digestibility corrected amino acid score (PDCAAS) of bread.

The IVPD and PDCAAS of the CFP enriched samples (Table 9.3C) ranged from 81.40% to 82.19% and 0.15 to 0.56, respectively. IVPD of bread fortified with CFP was significantly increased as compared to control. The pH drop curves obtained from enriched bread with CFP by using three enzyme (trypsin, α -chymotrypsin, and protease) system are shown in Figure 9.2.

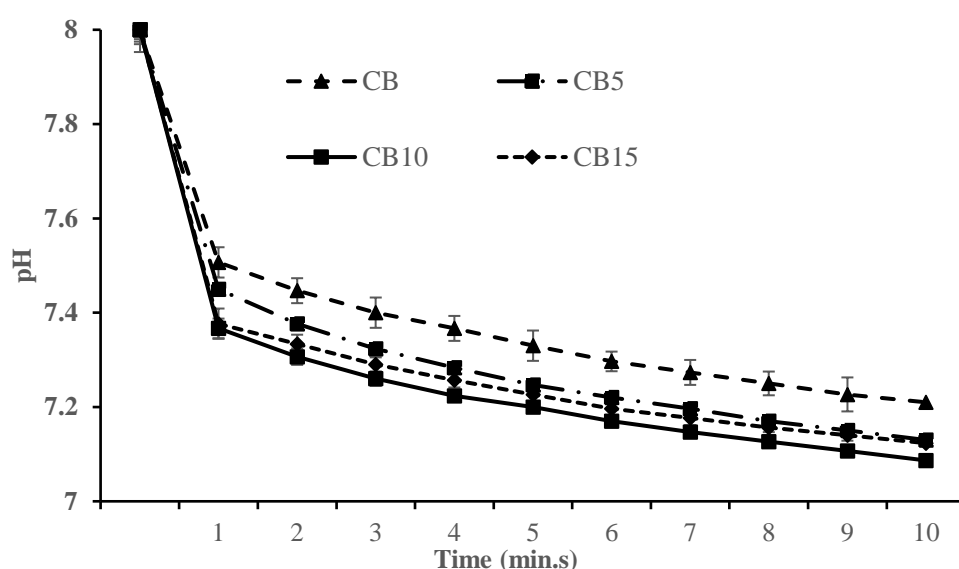


Figure 9.2 *In vitro* protein digestibility (pH vs Time) of bread enriched different levels of cod fish powder (CFP). CB, bread produced with semolina; CB5, CB10 and CB15, bread with 5,10, and 15 g CFP/100 g of cod powder. Results are the means of 3 measurements \pm standard deviations (n=3).

The drop in pH results from the release of amino acids and peptides, as proteins are digested. After addition of multi-enzymes to the protein solution, carboxyl ($-\text{COO}^-$) and amino ($-\text{NH}_3^+$) groups are released. At neutral pH (8.0), the free amino groups deionize in water and protons (H^+) are liberated. The free H^+ are released into the surrounding reaction medium causing a decrease in pH. Previous studies also demonstrated

increases in protein digestibility when lupin (*Lupinus angustifolius*) flour was added to wheat bread (Villarino, Jayasena, Coorey, Chakrabarti-Bell, Foley, et al., 2015a). Addition of CFP to bread significantly affect the IVPD that created a significant increase in the PDCAAS. These findings indicate that substitution of wheat flour with CFP at 5-15 g/100 g can potentially increase the IVPD and PDCAAS of wheat bread. This study is the first to report a significant IVPD value with the addition of CFP into bread formulation.

9.3.4 Glycaemic glucose equivalent (GGE) analysis of bread

The reducing sugar released during the *in vitro* digestion of bread fortified with CFP and control over 120 min is shown in Figure 9.3a.

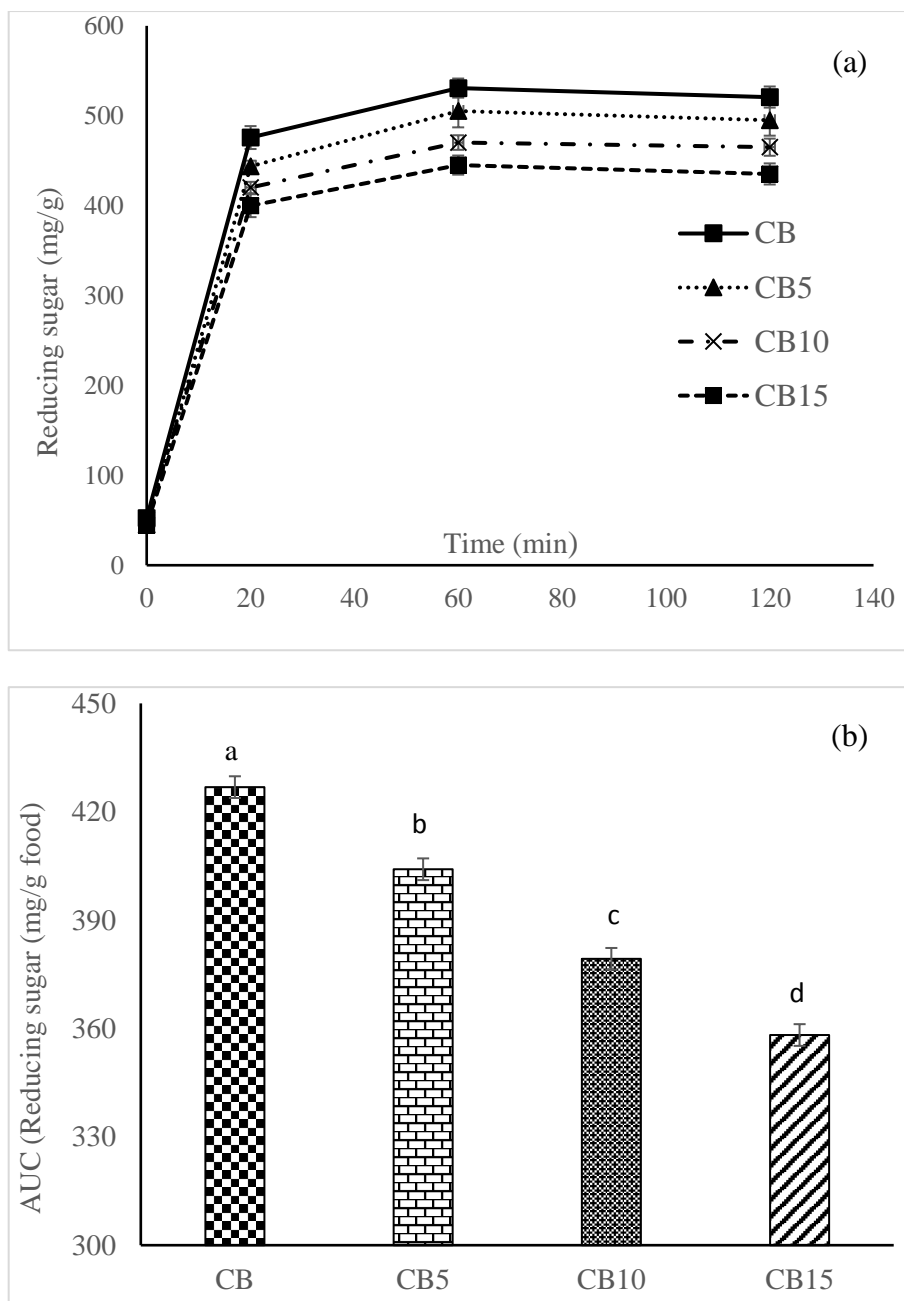


Figure 9.3 *In vitro* starch digestibility of bread. (a) Amount of reducing sugars released (mg/g food) during *in vitro* digestion. (b) Values for area under the curve (AUC) comparing the control bread. Control bread (CB); CB5, CB10 and CB15: bread produced with 5, 10 and 15 g cod fish powder/100 g semolina flour. Results are the mean of 3 measurements \pm standard deviations ($n=3$). ^{a to d} Values with different superscript letters differ significantly ($P < 0.05$).

It can be seen that there was a rapid starch degradation during the first 20 min. After 20 min, the starch was digested slowly between 20 and 120 min of digestion. There was a trend to reduce the starch degradation as the substitution level of CFP increased.

Bread with CFP at 10 and 15% levels revealed a significant decrease in the amount of reducing sugars released compared to the control sample, which could be attributed to higher amount of protein content in bread fortified with CFP that create physical barriers to limit the starch availability to starch hydrolysing enzymes. (Lu, Donner, & Liu, 2018; Reshmi et al., 2017) reported that bread fortified with pea flour and pomelo (*Citrus maxima*) fruit segments had a lower glycaemic index than control breads. The area under the glucose release curve is a measurement of glycaemic response for 2 h after the food is consumed (Brennan et al., 2012a). The values of area under the glycaemic response curve (AUC) shown in Figure 9.3b indicate that the addition of CFP to bread significantly decreased the AUC values with compared to control bread. Bread enriched with 5 to 15% CFP had the significantly lower AUC value compared to control bread (Figure 9.3b). According to previous research, there are several factors that influence the rate of starch digestion, such as type of starch, degree of starch gelatinisation, composition and structure, and non-starch components content of starch-protein matrix (Lu et al., 2018b). Liu et al. (2017b) have also found that wheat bran fibre can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzymes activity. Similar results were reported by Lu et al., (2018a), and bread fortified with pea flour had the lower glycaemic response due to the non-starch components and food matrix effects.

9.3.5 Total phenolic content and antioxidant activity

Consumption of food with phenolic rich ingredients is highly recommended due to their health promoting effects as they are involved in the prevention of many diseases such

as cancers, diabetes and cardiovascular diseases (Sayed Ahmad et al., 2018). The TPC of bread fortified with CFP and control are presented in Figure 9.4.

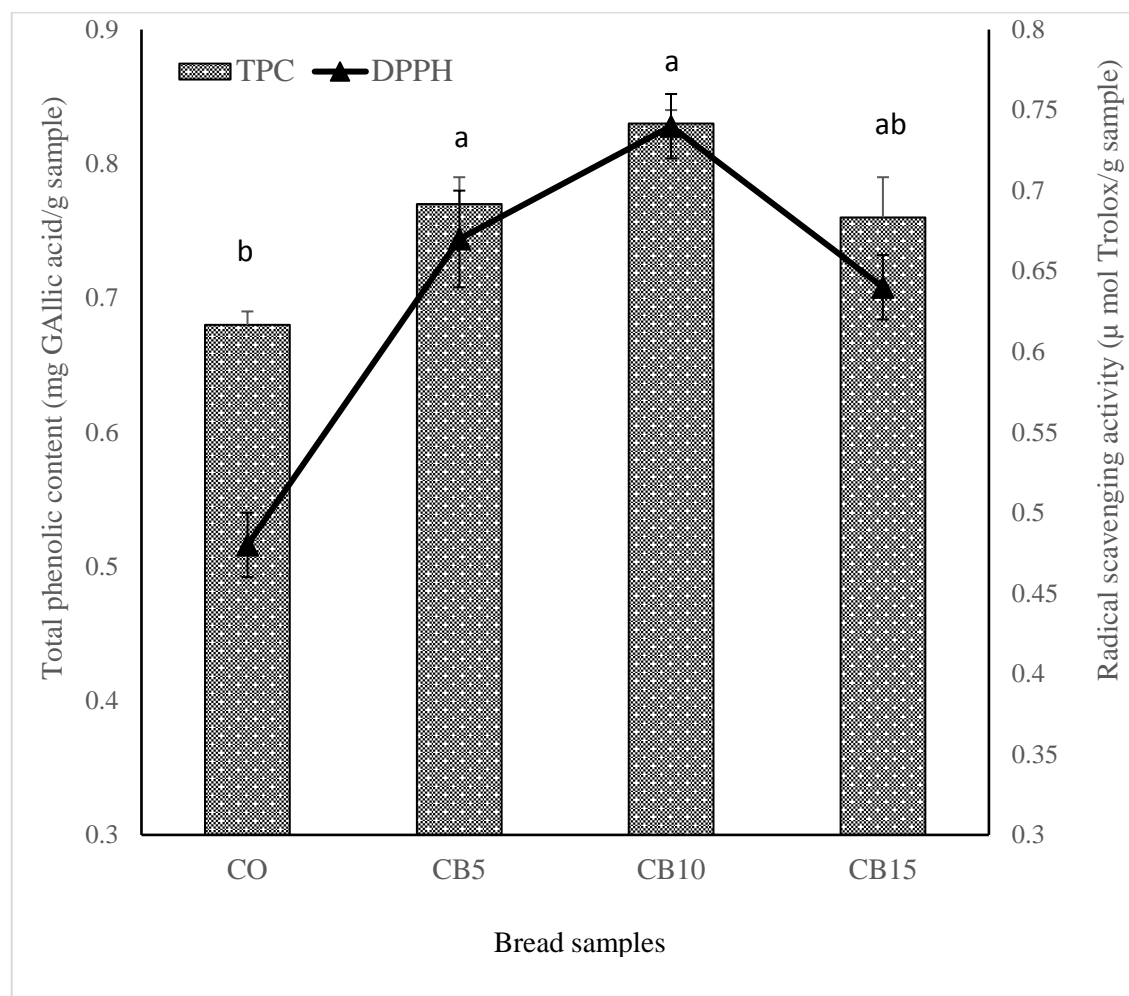


Figure 9.4 Total phenolic content (mg Gallic acid/g) and radical scavenging activity on DPPH radical of breads. CO, bread made with the semolina; CB5, CB10 and CB15, bread produced with 5%, 10% and 15% cod fish powder. Results are the mean of three measurements \pm standard deviations ($n=3$). ^{a to c} Values with different superscript letters differ significantly ($P < 0.05$).

Fortified bread samples had significantly higher TPC than the control bread. This increase in TPC can be attributed to the high content of phenol in added CFP, which agrees with previous studies such as the addition of lupin flour (Villarino, Jayasena, Coorey, Chakrabarti-Bell, Foley, et al., 2015a). The TPC of control bread was lower than the fortified bread due to the thermal deactivation of phenolic compound during baking

process. The total antioxidant activities (TEAC) of breads fortified with CFP are shown in Figure 9.4. The TEAC of enriched breads were significantly higher than those of the control bread. These results are in accordance with previous studies that reported the positive effect of bread fortification with lentil or carob flour on its antioxidant properties (Turfani et al., 2017). The correlation coefficients (R^2) of total phenolic content (TPC) and total antioxidant activity (TEAC) of the bread fortified with CFP was 0.97, which is in line with several previous studies (Sayed Ahmad et al., 2018).

9.4 Conclusion

This study demonstrated that fortification of bread with CFP is an effective technique to improve the physical, nutritional and nutraceutical potential of the final product. The addition of CFP significantly increased protein and decreased fat and moisture content of the bread as compared to control bread. The results indicate that inclusion of CFP in bread may be suitable to give acceptable volume, crumb characteristics, and textural properties. The phenolic content and antioxidant capacity of the bread were significantly enhanced by CFP inclusion. CFP fortified bread reduced starch digestion potentially by making a protein matrix mask around the starch granules thereby lowering the release of glucose. In comparison with the control bread the bread fortified with CFP exhibited a better amino acid profile and higher protein digestibility. These results indicated that CFP fortification might be a promising way to create a product with maximum potential nutritional and health benefits.

Chapter 10

Protein, amino acid, fatty acid composition, physical properties and *in vitro* starch and protein digestibility of bread fortified with salmon (*O. tschawytscha*) powder

This chapter is published as

Ajay S. Desai., Tang Beibeia, Margaret A. Brennan., Xinbo Guo, Xin-An Zeng & Charles S. Brennan. Protein, amino acid, fatty acid composition and *in vitro* digestibility of bread fortified with *Oncorhynchus tschawytscha*. *Nutrients*, **10**, 1923-1940

Abstract

This study investigated protein, amino acid, fatty acid composition, *in vitro* starch and protein digestibility, phenolic and antioxidant composition of bread fortified with salmon fish (*Oncorhynchus tschawytscha*) powder (SFP). The proximate composition in control and SFP breads ranged between (34-31.42 %) moisture, (13.91-20.04 %) protein, (3.86-9.13 %) fat, (2.13-2.42 %) ash, (80.10%-68.42%) carbohydrate and (410.8-435.96 kcal/100g) energy. The protein of control and SFP breads ranged between 261.75-306.96 mg/g protein which satisfies the score recommended by FAO/WHO/UNU (2007). The *in vitro* assay for protein digestibility, protein digestibility amino acid score, essential amino acid index, biological value and nutritional index ranged between 79.96-80.80 %, 0.15-0.42 %, 62.51-76.68 %, 56.44-71.68 %, 8.69-15.36 % respectively. Control and SFP breads contained 60.31-43.60 g/100g total fatty acids (saturated fatty acids) and 13.51-17.00 g/100g total fatty acids (polyunsaturated fatty acids) and SFP breads fulfil the ratio of $\omega 6/\omega 3$. There was a significant effect of SFP on bread specific volume, crumb colour, and textural properties. The *in vitro* starch digestibility results illustrate

that the incorporation of SFP into wheat bread decreased the potential glycaemic response of bread and increased the antioxidant capacity of bread. In conclusion, this nutrient rich SFP bread has the potential to be a technological alternative for the food industry.

10.1 Introdcuion

Fish is not only an excellent source of high nutritional value protein, but is an important source of essential amino acids (lysine, methionine and threonine) and lipid that contains ω -3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which relate to health benefits for humans such as prevention of cardiovascular disease, hypertension, cancer and diabetes as well as containing micronutrients such as vitamins (A, D, B6 and B12) and minerals (iron, zinc, iodine, selenium, potassium and sodium) (Dalton et al., 2009). DHA plays an important role in brain development and has the capacity to reduce the risk of cognitive function. The American heart association (AHA) recommends a minimum consumption of two fish serving per week (200mg/day of long chain ω -3 polyunsaturated fatty acid (PUFA) to achieve a cardio protective effect. Since EPA and DHA cannot be synthesised in the human body, they need to supply through the diet. To fulfil these requirements bread could be fortified with fish powder to achieve a balanced diet. Fish powder is rich in protein, amino acid and fatty acid which contributes to a low glycaemic index and has potential to have beneficial health effects such as manipulation of obesity, hypertension and cardiovascular disease (Kadam & Prabhasankar, 2010). Also, ω -3 enriched fish powder protects lipids from oxidation by CO_2 production during fermentation, especially while it is exposed to high temperatures during baking (Serna-Saldivar & Abril,

2011). Fish proteins are a good source of antioxidant activity in food products and can control health diseases by reduction of oxidative stress (Ahn, Kim, & Je, 2014). Bread is an important staple food worldwide, especially in western countries (Wandersleben et al., 2018). Western diets contain higher amounts of ω -6 than ω -3 fatty acids, which is not balanced for biological function. Bread is generally made of wheat flour, water, salt, sugar, yeast and fat. These products, are low in protein, vitamins and minerals and are usually deficient in essential amino acids and fatty acids and, therefore, are not a balanced food (Lu et al., 2018b). Due to its relatively low cost, availability, acceptability, and widespread consumption, bread is considered to be one the best vehicles for food fortification (Swieca, Gawlik-Dziki, Seczyk, Dziki, & Sikora, 2018). Fortification of a staple food is an effective way to create a product that can positively affect the consumer's health. Starch and protein are the major components of wheat bread and the incorporation of protein and lipid rich ingredients into the dough matrix affects the physical and nutritional properties of wheat bread by disturbing the viscoelastic network through the dilution of gluten structure (Graça, Fradinho, Sousa, & Raymundo, 2018). Proteins and amino acid derived from fish are considered nutritionally superior to that of plant origin ingredients. As consumer attention has become focused on the prevention of diseases such as cardiovascular disease, type-2 diabetes, colon cancer and obesity through diet (Freitas, Le Feunteun, Panouillé, & Souchon, 2018), there has been an increased demand for enrichment of food with improved physical (volume, colour and texture), nutritional (amino acid and fatty acid content, protein digestibility, starch digestibility) and antioxidant properties (Liu et al., 2017a). Low glycaemic index foods can be achieved through the utilisation of protein and lipid rich ingredients combined with cereal gains in products such as bread. Phenolic compounds exhibit

biological properties, such as antioxidant activity. Food rich in polyphenols have the potential to protect against various diseases associated with oxidative damage, such as cardiovascular, cancer and neurological disease (Sęczyk et al., 2016a). In recent years, to achieve this, many nutritional ingredients such as carob (Turfani et al., 2017), flaxseed and lupin (Wandersleben et al., 2018), mushroom powder (Lu et al., 2018a), cobia (Fagundes et al., 2018) and *Chlorella vulgaris* (Graça et al., 2018) have been incorporated into bread to improve its nutritional composition and product quality. Other products, such as pasta (Desai, Brennan, & Brennan, 2018) and pizza (El-Beltagi et al., 2017) have been developed with inclusion of fish powder in their formulation. However, the technological and nutritional properties of bread fortified with partial replacement of wheat flour by salmon fish (*O. tschawytscha*) powder (SFP) are still unknown. Therefore, this study evaluated the effects of fortification of different levels SFP on the bread nutritional quality including protein, amino acid, fatty acid composition and *in vitro* starch and protein digestibility; the physical characteristics, its technological and antioxidant properties of bread.

10.2 Materials and method

10.2.1 Raw materials

Described in section 3.1

10.2.2 Fish powder preparation

Described in section 3.1.1

10.2.3 Preparation of bread

Described in section 3.2.3

10.2.4 Proximate chemical composition analysis of SFP enriched bread

Described in section 3.4.1 to 3.4.4

10.2.5 Volume, density, moisture and texture properties of bread

Described in section 3.4.11.6

10.2.6 Coloure measurements

Described in section 3.4.13.4

10.2.7 *In vitro* starch digestibility of bread

Described in section 3.4.6

10.2.8 Amino acid profile and scoring

Described in section 3.4.7

10.2.9 *In vitro* protein digestibility, protein digestibility corrected amino acid score (PDCAAS) and nutritional index.

Described in section 3.4.5

10.2.10 Fatty acid profile of bread fortified with SFP

Described in section 3.4.9

10.2.11 Total phenolic content and antioxidant activity of bread

Described in section 3.4.12.1 and 3.4.12.2

10.2.12 Statistical analysis

Described in section 3.4.22

10.3 Results and discussion

10.3.1 Chemical composition and physical properties of bread

The chemical composition and physical properties of breads enriched with different levels of SFP are presented in Table 10.1. The protein content of bread enriched with SFP ranged from 16.27 to 20.04 % compared to 13.91 % in the control bread. The

inclusion of 5-15 % SFP to the formulations increased ($P < 0.05$) the levels of protein and fat content and decreased ($P < 0.05$) the carbohydrate content (75-68 %) compared to those of the control bread.

Table 10.1 Bread formulations, proximate composition (on an as-is basis) and physical properties of bread. A. Ingredients used in salmon powder (SP) enriched bread. B. Proximate composition of bread elaborated with SP. C. Physical properties of bread made with SP.

A						
Sample code						
Ingredients (g)	Control	5% SFP	10% SFP	15% SFP		
Wheat flour	150	142.5	135	127.5		
Salmon powder	-	7.5	15	22		
salt	2.25	2.25	2.25	2.25		
Sugar	9	9	9	9		
Yeast	2.25	2.25	2.25	2.25		
Butter	7.5	7.5	7.5	7.5		
Water	90	90	90	90		
B						
Sample code	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	Energy (Kcal)
Control	13.91 ± 0.19 ^d	3.86 ± 0.02 ^d	2.13 ± 0.02 ^b	34.38 ± 0.55 ^a	80.10 ± 0.18 ^a	410.8 ± 0.18 ^d
5% SFP	16.27 ± 0.08 ^c	6.03 ± 0.06 ^c	2.36 ± 0.05 ^a	31.42 ± 0.42 ^a	75.35 ± 0.11 ^b	420.71 ± 0.17 ^c
10% SFP	18.22 ± 0.06 ^b	7.26 ± 0.08 ^b	2.42 ± 0.09 ^a	32.90 ± 0.47 ^a	72.10 ± 0.19 ^c	426.62 ± 0.44 ^b
15% SFP	20.04 ± 0.10 ^a	9.13 ± 0.02 ^a	2.42 ± 0.09 ^a	33.33 ± 0.73 ^a	68.42 ± 0.11 ^d	435.96 ± 0.36 ^a
C						
Sample	Width/ height ratio		Volume (mL)	Specific volume (mL/g)		
Control	1.80 ± 0.12 ^a		111 ± 1.00 ^a	2.47± 0.02 ^c		
5% SFP	1.77 ± 0.38 ^b		103 ± 1.15 ^b	2.35 ± 0.01 ^b		
10% SFP	1.74 ± 0.15 ^b		97 ± 0.58 ^c	2.18 ± 0.00 ^a		
15% SFP	1.77 ± 0.10 ^b		96 ± 1.05 ^c	2.16 ± 0.02 ^a		

5% SFP, 10% SFP and 15% SFP: bread prepared with 5, 10 and 15g of salmon fish powder /100 g of wheat flour. Control bread. (n=3 ± standard deviation).

Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

The lipid content of SFP enriched breads varied from 6.03 to 9.13 % compared to 3.86 % in control bread. The 5-15 % SFP supplemented bread exhibited significantly increased lipid content compared to the control bread. The ash contents of SFP breads ranged from 2.36 to 2.42 % which were different ($P > 0.05$) compared to control bread. The low moisture content was observed in SFP enriched bread compared to the control bread. Lower moisture content in SFP enriched bread may be due to the protein-polysaccharides interaction and low water retention capacity compared to control bread. Previous research has shown an increase in protein, lipid content and decreased carbohydrate and moisture contents when cobia powder and flaxseed and lupin were added to bread formulations, respectively (Fagundes et al., 2018; Wandersleben et al., 2018). The energy value obtained in SFP enriched breads was higher ($P < 0.05$) than the control bread. Calculated lipid contents in SFP enriched bread shown that there was increase in content. Similar results were obtained by (Sayed Ahmad et al., 2018) for bread with added cumin and caraway flour powder.

The incorporation of SFP decreased bread volume from 111 to 96 mL and specific volume from 2.47 to 2016 mL/g (Table 10.1C). The width/height ratio of bread enriched with SFP decreased in comparison with control bread. It is known that gluten is the major visco-elastic protein which helps to shape the bread by trapping fermentation gases, the decrease in volume could be due to the reduction in gluten content. Such a dilution of gluten and the interactions of SFP protein and lipid in the gluten matrix during the processing of the breads may reduce the ability of SFP breads to extend and hold the carbon dioxide produced during fermentation. Our finding is in agreement with the results of (Fagundes, et al., 2018) who prepared bread enriched with cobia (*Rachycentron canadum*) flour and found that increasing the cobia flour levels in wheat

bread decreased the bread volume. The effect of flaxseed hull on bread volume has been studied and was found to progressively decrease the loaf volume as the level of flaxseed protein and lipid increased (Seczyk et al., 2017). In addition, the different nutrient composition had different effects on the gas production during the fermentation process of bread, thus influencing bread volume (Lu et al., 2018a).

10.3.2 Texture and colour measurement of bread

The textural properties of bread, shown in table 10.2A and Figure 10.1, are important parameters to consider to ensure consumer acceptance, they are mainly controlled by the gluten network. The control bread had the lowest hardness, fortification with 5 % and 10 % SFP caused a significant increase in hardness whereas 15% SFP was harder than the control bread but not significant. The hardness of bread is related to the peak force required to compress the sample, while chewiness represents a quantitative estimation of energy needed to disintegrate bread structure (Liu et al., 2017a).



Figure 10.1 Texture analyser with probe for measurement of texture quality of bread.

Bread hardness was influenced by SFP which may be attributed to the thickening of gas cell walls within the bread crumb due to the high water absorption characteristics of SFP protein. Additionally, increased protein and lipid content in SFP bread may decrease

gas retention causing unstable gas cells resulting in a more compact structure. This result was consistent with the work of (Fagundes, et al., 2018; Vijaykrishnaraj et al., 2016) who reported that inclusion of cobia fish protein and green mussel (*Perna canaliculus*) resulted in a significant ($P < 0.05$) increase of bread firmness.

Table 10.2 Technological characteristic of bread enriched with different levels of salmon powder (SB). A. Texture profile analysis. B. Colour characteristics of crust and crumb.

A				
Parameter	Control	5% SFP	10% SFP	15% SFP
Hardness (g)	1082.77 ± 0.20 ^b	1648.48 ± 44.88 ^a	1525.91 ± 60.81 ^a	1198.41 ± 78.59 ^b
Springiness(mm)	0.935 ± 0.03 ^a	0.892 ± 0.01 ^b	0.882 ± 0.03 ^b	0.879 ± 0.01 ^b
Gumminess (g)	788.99 ± 5.01 ^b	946.54 ± 5.26 ^a	962.11 ± 86.19 ^a	776.15 ± 33.23 ^b
Chewiness (g)	737.25 ± 10.79 ^b	835.58 ± 18.70 ^a	886.89 ± 76.60 ^a	674.79 ± 42.26 ^c
Cohesiveness	0.73 ± 0.01 ^a	0.58 ± 0.02 ^b	0.62 ± 0.02 ^b	0.62 ± 0.02 ^b
Resilience	0.35 ± 0.01 ^a	0.27 ± 0.01 ^b	0.29 ± 0.02 ^b	0.29 ± 0.01 ^b
B				
Sample code	Crust colour			ΔE
	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	
Control	91.45 ± 0.32 ^a	9.97 ± 0.27 ^a	36.65 ± 0.07 ^a	-
5% SFP	85.46 ± 0.11 ^b	2.17 ± 0.63 ^b	32.57 ± 0.09 ^b	10.67 ± 0.34 ^a
10% SFP	86.05 ± 0.38 ^b	1.58 ± 0.37 ^{bc}	33.42 ± 0.91 ^b	10.49 ± 0.34 ^a
15% SFP	85.95 ± 0.22 ^b	0.66 ± 0.23 ^c	33.73 ± 0.41 ^b	11.26 ± 1.08 ^a
Sample code	Crumb colour			ΔE
	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	
Control	95.31 ± 0.12 ^b	12.23 ± 0.02 ^c	30.92 ± 0.08 ^d	-
5% SFP	96.54 ± 0.11 ^a	12.96 ± 0.23 ^b	32.55 ± 0.56 ^c	2.23 ± 0.69 ^a
10% SFP	96.59 ± 0.16 ^a	13.23 ± 0.12 ^{ab}	33.92 ± 0.26 ^b	3.41 ± 0.27 ^b
15% SFP	96.31 ± 0.06 ^a	13.53 ± 0.12 ^a	35.54 ± 0.03 ^a	4.89 ± 0.23 ^c

5% SFP, 10% SFP and 15% SFP: bread prepared with 5, 10 and 15g of salmon fish powder /100 g of wheat flour. Control bread. (n=3 ± standard deviation).

Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

A significant ($P < 0.05$) increase were observed in gumminess and chewiness, of SFP enriched breads compared to the control bread (except at the 15 % level). Similar

results have been noted in bread substituted by faba bean flour, where there was decrease in gumminess and chewiness by such inclusion (Turfani et al., 2017).

However, springiness, cohesiveness, and resilience of bread supplemented with SFP were decreased ($P < 0.05$) compared to control bread. Springiness represents the capacity of sample to spring back after a deformation due to the compression. Similar results were found by (Lu et al., 2018a), who reported that addition of mushroom powder led to lower bread springiness and cohesiveness value.

The lightness (L^*), redness (a^*), and yellowness (b^*) values of crust and crumb of bread enriched with SFP are shown in Table 10.2B and Figure 10.2.



Figure 10.2 Bread enriched with different levels of salmon powder.

As the levels of SFP increased, the L^* , a^* , b^* of bread crust decreased. Bread fortified with 20% SFP provided the lowest L^* , a^* , b^* . For the bread crumb, the L^* , a^* , b^* values increased as the level of SFP increased. Bread colour is the result of complex reactions that depend on the physicochemical characteristics of dough (water, starch and lysine content) and the temperature used during baking process. The addition of SFP alters the crumb colour to be more yellowish (b^*). The darkening of SFP breads may also be

attributed to an increased Maillard reaction taking place during baking due to the higher lysine content. In the Maillard reaction reducing carbohydrates react with free amino acid side chain of protein mainly lysine and lead to amino acid-sugar reaction products (Turfani et al., 2017). The results obtained by Sanz-Penella et al. (2013) are similar to the observation of low and high L^* and b^* values in bread crust and crumb respectively. They reported that the lightness and yellowness values of bread crust and crumb increased as the level of amaranth flour (10-40 %) powder increased in bread. Also, Turfani et al. (2017) demonstrated that incorporation of 6, 12 and 24 % lentil flour in bread significantly affected yellowness and lightness characteristics. The ΔE values were also determined to evaluate the colour differences between the control and the SFP formulations. The ΔE values of bread crust and crumb increased ($P < 0.05$) with increasing levels of SFP. In addition, the bread crust exhibited higher ΔE compared to the bread crumb, which indicative of the colour compounds created as a result of baking. The ΔE values were more than 3.0 for bread crust and crumb. According to handbook of colour science, these values fall in the “appreciable, detectable by ordinary people”. Reshmi et al. (2017) reported that pomelo enriched bread showed higher ΔE value in bread crust as inclusion increased from 2-7.5%.

10.3.3 Protein quality of bread

10.3.3.1 Amino acid profile and amino acid scoring of bread samples

Protein quality is considered one of the important characteristics for measuring the nutritional characteristic of a food matrix. SFP inclusion increased ($P < 0.05$) the concentration of essential amino acids (EAA) such as lysine, leucine, isoleucine, methionine, tyrosine, threonine, and valine (Table 10.3A).

Table 10.3 Amino acid (AAs) composition (mg/g protein dry weight basis) and nutritional characterisation of wheat bread (control bread) and breads enriched with different levels of salmon fish powder. IVPD: *in vitro* protein digestibility; EAAI: Essential Amino Acid Index, BV: biological value, NI Nutritional Index.

A				
Amino acid	Control	5% SFP	10% SFP	15% SFP
Phenylalanine	42.24 ± 0.19 ^a	38.12 ± 2.30 ^{ab}	40.33 ± 2.50 ^{ab}	37.48 ± 0.92 ^b
Tyrosine	18.66 ± 2.08 ^b	23.62 ± 1.20 ^{ab}	25.81 ± 3.37 ^a	26.47 ± 0.33 ^a
Histidine	20.75 ± 1.33 ^a	17.79 ± 1.12 ^a	20.42 ± 0.89 ^a	19.42 ± 0.75 ^a
Isoleucine	30.31 ± 0.81 ^b	30.63 ± 1.84 ^b	34.58 ± 1.87 ^a	34.64 ± 0.68 ^a
Leucine	62.18 ± 0.57 ^a	61.13 ± 3.68 ^a	68.25 ± 3.72 ^b	66.50 ± 1.41 ^b
Lysine	9.69 ± 1.14 ^c	16.42 ± 1.10 ^b	23.76 ± 1.68 ^a	26.94 ± 1.00 ^a
Methionine	10.88 ± 0.65 ^c	13.71 ± 0.63 ^b	16.93 ± 1.19 ^a	17.42 ± 0.22 ^a
Cysteine	11.69 ± 0.98 ^a	10.35 ± 0.57 ^{ab}	9.56 ± 0.61 ^b	8.64 ± 0.52 ^b
Threonine	23.22 ± 0.22 ^b	24.46 ± 1.62 ^b	29.78 ± 1.36 ^a	30.52 ± 0.59 ^a
Valine	32.17 ± 0.90 ^b	32.86 ± 1.93 ^b	37.50 ± 2.07 ^a	37.57 ± 0.71 ^a
ΣEAAs	261.75 ± 9.23^a	269.14 ± 8.69^b	306.96 ± 7.89^c	305.65 ± 6.76^c
B. Amino acid score^a				
Histidine	1.15 ± 0.08 ^a	0.99 ± 0.06 ^a	1.13 ± 0.04 ^a	1.07 ± 0.04 ^a
Isoleucine	0.98 ± 0.03 ^b	0.99 ± 0.05 ^b	1.12 ± 0.06 ^a	1.12 ± 0.02 ^a
Leucine	0.99 ± 0.01 ^a	0.97 ± 0.05 ^a	1.08 ± 0.05 ^a	1.05 ± 0.02 ^a
Lysine	0.19 ± 0.02 ^c	0.32 ± 0.02 ^b	0.46 ± 0.03 ^a	0.52 ± 0.01 ^a
Phenylalanine + Tyrosine	1.32 ± 0.05 ^a	1.34 ± 0.05 ^a	1.43 ± 0.06 ^a	1.39 ± 0.02 ^a
Methionine + Cysteine	0.87 ± 0.06 ^b	0.92 ± 0.02 ^{ab}	1.01 ± 0.01 ^a	1.00 ± 0.03 ^a
Threonine	0.86 ± 0.02 ^b	0.91 ± 0.06 ^b	1.10 ± 0.05 ^a	1.13 ± 0.02 ^a
Valine	0.77 ± 0.03 ^b	0.78 ± 0.04 ^b	0.89 ± 0.04 ^a	0.89 ± 0.01 ^a
C. IVPD, PDCAAS and nutritional Index				
IVPD (%)	79.96 ± 0.65 ^a	80.80 ± 0.99 ^a	80.20 ± 0.10 ^a	80.60 ± 0.73 ^a
PDCAAS ^b	0.15 ± 0.06 ^a	0.26 ± 0.05 ^b	0.37 ± 0.02 ^c	0.42 ± 0.06 ^d
EAAI	62.51 ± 1.15 ^a	71.44 ± 1.28 ^b	76.76 ± 1.95 ^c	76.68 ± 1.40 ^c
BV	56.44 ± 1.05 ^a	66.18 ± 1.22 ^b	71.97 ± 1.56 ^c	71.68 ± 1.10 ^c
NI	8.69 ± 0.10 ^a	11.62 ± 0.19 ^b	13.98 ± 0.38 ^c	15.36 ± 0.21 ^d

Bold value indicate the grand totals for Essential amino acids (ΣEAAs). 5% SFP, 10% SFP and 15% SFP: bread prepared with 5, 10 and 15 g of salmon fish powder /100 g of wheat flour. Control bread. (n=3 ± standard deviation). ^a Based on standard FAO/WHO/UNU (2007) 1-2 year old reference pattern (mg/g protein): Histidine-18; Lysine-52; Isoleucine-31; Leucine-63; Methionine + Cysteine-26; Phenylalanine + Tyrosine-46; Threonine-27; Valine-42.

^b Protein digestibility corrected amino acid score (PDCAAS): AAS (lowest score of an individual amino acid) x *In vitro* protein digestibility of bread sample

Values within a column followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test

Lysine (69-178 %), methionine (26-60 %), tyrosine (27-42 %) and threonine (5-32 %) increased in a manner ($P < 0.05$) dependent on 5-15% SFP inclusion compared to the control bread. However, the levels of isoleucine, leucine and valine increased more than expected ($P < 0.05$) with 10 % and 15 % SFP inclusions. The contents of phenylalanine and cysteine decreased in SFP bread compared to the control. No difference was observed in histidine content. Bread prepared with different levels of SFP (SFP 15 % > SFP10 % > SFP5 %) exhibited greater ($P < 0.05$) total EAA contents than the control. (Coda et al., 2017) reported a similar pattern for total EAA levels in bread enriched with faba bean.

The SFP bread, had a significantly ($P < 0.05$) higher lysine, isoleucine, leucine, methionine + cysteine, threonine and valine score (Table 10.3a). There was no significant difference in phenylalanine + tyrosine and histidine scores compared to the control bread. The scores for isoleucine, leucine, lysine, methionine cysteine, threonine and valine in SFP bread were higher than the standards for amino acids of ideal reference protein, appropriate for children ages 1–2 (which also covers the range appropriate for human adults) (FAO/WHO/UNU, 2007). Based on the essential amino acid scores (Table 3B), the limiting amino acid is lysine for all bread, therefore the scores for lysine were used to calculate the PDCAAS of the breads. A higher level of SFP addition would, therefore, be required to further improve the lysine score of the bread.

10.3.3.2 *In vitro* protein digestibility (IVPD), protein digestibility corrected amino acid score (PDCAAS) and nutritional index of bread.

The IVPD and PDCAAS of the SFP bread (Table 10.3C) ranged from 80.80 % to 80.60 % and 0.26 to 0.42, respectively. IVPD of SFP bread was higher than the control bread.

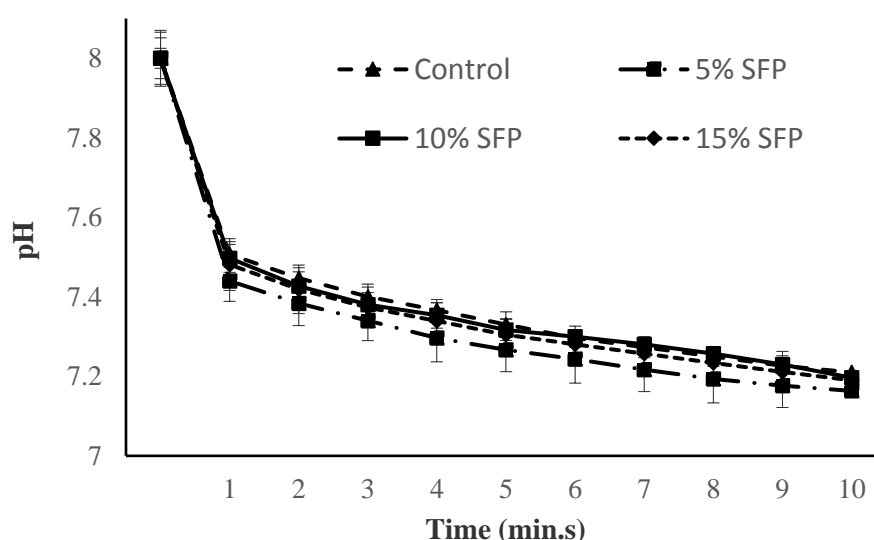


Figure 10.3 *In vitro* protein digestibility of bread enriched different levels of salmon fish powder (SFP). Control bread; 5% SFP, 10% SFP and 15% SFP: bread produced with 5, 10 and 15 g salmon fish powder/100 g wheat flour. (n=3 ± standard deviation).

The pH drop curves of SFP bread using the three enzyme (trypsin, α -chymotrypsin, and protease) system are shown in Figure 10.3. Previous studies also demonstrated increases in protein digestibility when legume lupin (*Lupinus angustifolius*) flour was added to wheat bread (Villarino, Jayasena, Coorey, Chakrabarti-Bell, Foley, et al., 2015a). Addition of SFP to bread significantly affected the IVPD that translated to a significant increase in the PDCAAS. These results indicate that substitution of wheat flour with SFP at 5-15 g/100 g can potentially increase the IVPD and PDCAAS of wheat bread. This study is the first to report a significant change to IVPD value with the

addition of SFP to bread. Compared to control bread, EAAI and BV were significantly ($P < 0.05$) higher for SFP bread. Among the indices that are used to evaluate the nutritional value of foods, NI combines qualitative and quantitative factors and it is considered as global predictor of the protein quality (Lorusso et al., 2017). Since the protein bioavailability increased, the NI value of SFP breads was significantly ($P < 0.05$) higher than the control bread (Table 10.3C). Similarly wheat bread enriched with faba bean had significantly ($P < 0.05$) increased EAAI, BV and NI indices (Coda et al., 2017).

10.3.4 Fatty acid profile of bread

SFP bread had lower ($P < 0.05$) SFA and total SFA than the control bread (Table 10.4). The replacement of SFA by unsaturated fats in the food product reduces the risk of developing cardiovascular diseases (Coelho & Salas-Mellado, 2015).

Table 10.4 Fatty acid profile (g of individual fatty acids/100g of total fatty acids) of bread enriched with different levels of salmon fish powder (SFP).

Fatty acid	Control	5% SFP	10% SFP	15% SFP
Saturated Fatty Acids (SFA)				
C12:0	3.68 ± 0.01 ^a	2.69 ± 0.02 ^b	1.99 ± 0.01 ^c	1.70 ± 0.00 ^d
C14:0	9.74 ± 0.06 ^a	7.64 ± 0.07 ^b	6.11 ± 0.04 ^c	5.56 ± 0.01 ^d
C15:0	1.77 ± 0.01 ^a	1.36 ± 0.01 ^b	1.08 ± 0.01 ^c	0.95 ± 0.01 ^d
C16:0	35.48 ± 1.44 ^a	31.87 ± 0.08 ^b	29.14 ± 0.05 ^c	28.01 ± 0.03 ^d
C17:0	0.96 ± 0.08 ^a	0.75 ± 0.08 ^b	0.62 ± 0.04 ^{bc}	0.56 ± 0.04 ^c
C18:0	8.27 ± 0.11 ^a	7.25 ± 0.10 ^b	6.50 ± 0.01 ^c	6.20 ± 0.01 ^d
C19:0	0.29 ± 0.01 ^a	0.20 ± 0.01 ^b	0.15 ± 0.01 ^b	0.19 ± 0.01 ^b
C20:0	0.11 ± 0.01 ^a	0.12 ± 0.00 ^a	0.12 ± 0.00 ^a	0.12 ± 0.00 ^a
C22:0	-	0.07 ± 0.00 ^c	0.14 ± 0.01 ^b	0.16 ± 0.01 ^a
C24:0	-	0.06 ± 0.01 ^b	0.11 ± 0.02 ^a	0.13 ± 0.01 ^a
Monounsaturated Fatty Acids (MUFA)				
C14:1	0.83 ± 0.01 ^a	0.63 ± 0.00 ^b	0.50 ± 0.00 ^b	0.43 ± 0.00 ^d
C16:1 ω-7	1.37 ± 0.02 ^d	2.52 ± 0.01 ^c	3.31 ± 0.01 ^b	3.73 ± 0.00 ^a
C17:1	0.14 ± 0.01 ^b	0.17 ± 0.00 ^a	0.18 ± 0.01 ^a	0.18 ± 0.00 ^a
C18:1 ω-9	17.28 ± 0.08 ^d	23.67 ± 0.12 ^c	28.41 ± 0.01 ^b	31.09 ± 0.04 ^a
C20:1	0.03 ± 0.04 ^d	0.46 ± 0.03 ^c	0.88 ± 0.02 ^b	1.05 ± 0.01 ^a

C22:1 ω-9	-	0.10 ± 0.00 ^c	0.15 ± 0.01 ^b	0.17 ± 0.00 ^a
C24:1 ω-9	-	-	0.07 ± 0.00 ^b	0.08 ± 0.01 ^a
Polyunsaturated fatty acids (PUFA)				
C18:2 ω-6	12.08 ± 0.09 ^c	12.68 ± 0.06 ^b	12.97 ± 0.04 ^a	12.91 ± 0.01 ^c
C18:3 ω-3	1.35 ± 0.00 ^d	1.40 ± 0.01 ^c	1.50 ± 0.01 ^b	1.45 ± 0.00 ^a
C20:2 ω-6	-	0.10 ± 0.01 ^c	0.15 ± 0.00 ^b	0.17 ± 0.00 ^a
C20:3 ω-6	-	0.09 ± 0.01 ^c	0.14 ± 0.00 ^b	0.17 ± 0.00 ^a
C20:4 ω-6	-	0.14 ± 0.01 ^c	0.22 ± 0.01 ^b	0.25 ± 0.00 ^a
C20:5 ω-3	-	0.29 ± 0.00 ^c	0.46 ± 0.00 ^b	0.58 ± 0.01 ^a
C22:2 ω-6	-	-	-	0.04 ± 0.01
C22:5 ω-3	0.09 ± 0.04 ^d	0.18 ± 0.01 ^c	0.25 ± 0.00 ^b	0.28 ± 0.00 ^a
C22:6 ω-3	-	0.78 ± 0.03 ^c	1.31 ± 0.01 ^b	1.54 ± 0.01 ^a
ΣSFA	60.31 ± 0.45 ^a	49.33 ± 0.41 ^b	43.96 ± 0.27 ^c	43.60 ± 0.12 ^c
ΣMUFA	19.65 ± 0.17	27.56 ± 0.18	33.50 ± 0.12	36.75 ± 0.06
ΣPUFA	13.51 ± 0.14	15.68 ± 0.13	17.00 ± 0.08	16.62 ± 0.04
Σ ω-6	12.08 ± 0.01	13.01 ± 0.02	13.48 ± 0.05	12.83 ± 0.01
Σ ω-3	1.43 ± 0.01	2.66 ± 0.04	3.52 ± 0.03	3.80 ± 0.02
PUFA/SFA	0.22	0.31	0.38	0.38
EPA+DHA	-	1.07 ± 0.03	1.77 ± 0.01	2.06 ± 0.01
ω-6/ ω-3	12.08	4.89	3.82	3.37

5% SFP, 10% SFP and 15% SFP: Bread prepared with 5, 10, and 15 g of salmon fish powder /100 g of wheat flour. Control bread sample. (n=3 ± standard deviation).

Values within a row followed by the same superscript letter are not significantly different from each other ($P > 0.05$), according to Tukey's test.

SFP fortification increased the content of palmitoleic (C16:1), oleic acid (C18:1) and gadoleic acid (C20:1) compared to the control bread and resulted in an increase ($P < 0.05$) of total monounsaturated fatty acid (MUFA) content in SFP bread. The major PUFA present in control bread was linoleic acid (C18:2). ω-3 fatty acids were low in content with the ratio of ω-6: ω-3 being 12.08:1. This predominance of n-6 linoleic acid in cereal is a major contribution to the imbalanced ω-6: ω-3 ratio (Fradique et al., 2013). Increasing the percentage of SFP significantly enhanced EPA and DHA fatty acid content. In turn, the bread prepared with SFP presented an important proportion of this long chain unsaturated fatty acid in relation to other fatty acids. Even in the bread made with

5 % SFP, it was possible to obtain an important reduction of the ratio ω -6: ω -3 (4.89:1) in comparison with the control bread. From the human nutrition standpoint and prevention of cardiovascular disease, a diet with an ω -6/ ω -3 ratio between 1 and 5 is recommended by food agencies, scientific societies and national and international organisations (Agostoni et al., 2010). Similar findings were observed by Coelho & Salas-Mellado, (2015), who reported that inclusion of chia flour to wheat bread significantly decreased SFA and increased PUFA. Research has indicated that inclusion of shrimp meat (*Penaeus monodon*) and carp fish powder (*Cyprinus carpio*) could raise this ratio (El-Beltagi et al., 2017; Ramya et al., 2015). The recommendations of EPA and DHA are 0.250-2 g/ day. The consumption of 100 g SFP bread provides 1.07 to 2.06 g of EPA and DHA, which fulfils the minimum daily value recommended. Polyunsaturated fatty acids are easily oxidised in baking processes affecting their stability, however, a significant amount of EPA and DHA remained stable in the bread product. Future studies about the effects of the baking processes, of the bread on EPA and DHA content are necessary.

10.3.5 *In vitro* starch digestion analysis

Protein, lipid and starch play an important role in starch digestibility and in the glycaemic response in the human body (Ren et al., 2016). It can be seen that there were significantly ($P < 0.05$) more reducing sugars released from the control bread than from the SFP fortified breads at 20 min of time digestion (Figure 10.4a).

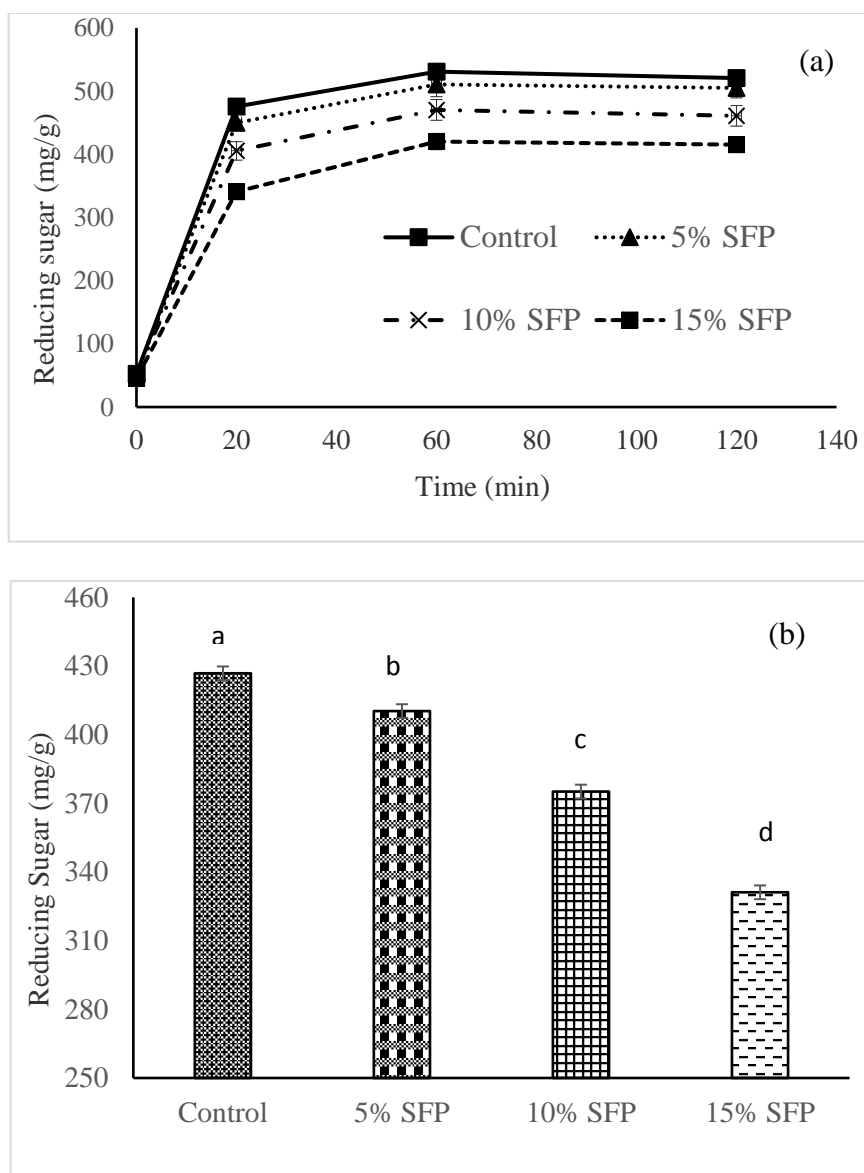


Figure 10.4 *In vitro* starch digestibility of bread. (a) Amount of reducing sugars released (mg/g starch) during *in vitro* digestion. (b) Values for are under the curve (AUC) comparing the control bread. Control bread; 5% SFP, 10% SFP and 15% SFP: bread produced with 5, 10 and 15 g salmon fish powder, 100 g wheat flour. (n=3 ± standard deviation). ^{a to d} Values with different superscript letters differ significantly ($P < 0.05$).

The strongest decrease was observed after addition of 10 and 15 % SFP followed by 5 % SFP bread samples which could be attributed to higher amount of protein and lipid content in bread fortified with SFP compared to control bread that formed amylose-lipid complex which limit the starch availability to starch hydrolysing enzymes. SFP bread showed significantly higher oleic acid, gadoleic acid, linoleic and α -linoleic acid

compared to control bread (Table 10.4). The amylose-lipid interactions results in the formation of single helical structure with a conformational hindrance that restricts enzymes trying to hydrolyse the starch granule. Similar results were reported when investigating the *in vitro* starch digestibility and glycaemic index of millets with different fatty acids palmitic, oleic and linoleic acids (Annor et al., 2015). Additionally, protein rich SFP reduced starch granule surface accessibility and therefore influenced the enzyme susceptibility. It was previously reported that the presence of protein content in food matrix creates a strong network and reduces the capacity of enzyme attack to the starch granules, thereby delaying starch digestion (Ren et al., 2016). Reshmi et al., 2017 reported that bread fortified with pea flour and pomelo (*Citrus maxima*) fruit segments had a lower glycaemic index than control breads. The area under the glucose release curve is a measurement of glycaemic response for 2 h after food consumed (Brennan et al., 2012a). The values of area under the predictive glycaemic response curve (AUC) shown in Figure 10.4b indicate that the addition of SFP to bread significantly decreased the AUC values with compared to control bread. Bread enriched with 5 to 15 % SFP had the significantly lower AUC value compare to control bread (Figure 10.4b). According to previous research, there are several factors that influence the rate of starch digestion, such as type of starch, degree of starch gelatinisation, composition and structure, and non-starch components content of starch-protein matrix (Lu et al., 2018b). Similar results were reported by Lu et al., (2018a) in which bread fortified with pea flour had a lower glycaemic response due to the non-starch components and food matrix effects.

10.3.5 Total phenolic content and antioxidant activity of bread

Consumption of food with phenolic rich ingredients is highly recommended due to their health promoting effects as they are involved in the prevention of many diseases such as cancers, diabetes and cardiovascular diseases (Sayed Ahmad et al., 2018). SFP bread had lower TPC values than the control bread (Figure 10.5).

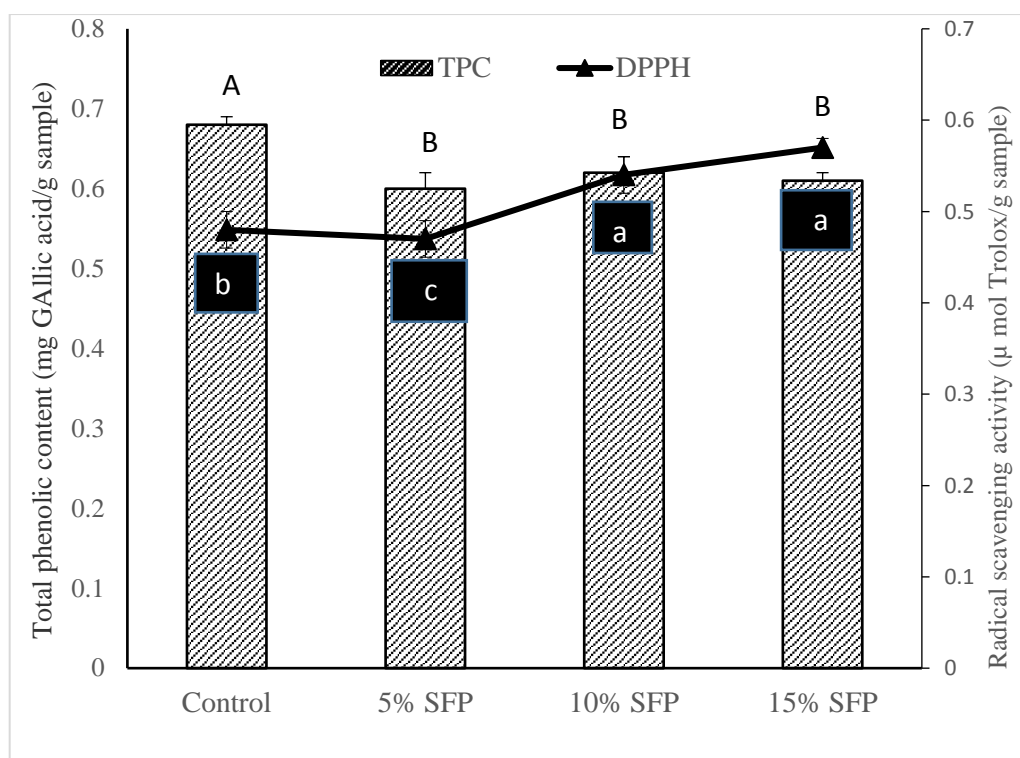


Figure 10.5 Total phenolic content (mg Gallic acid/g) and radical scavenging activity on DPPH radical of breads. Control bread; 5% SFP, 10% SFP and 15% SFP: bread produced with 5, 10 and 15 g salmon fish powder, 100 g wheat flour. (n=3 ± standard deviation). ^{a to c} Values with different superscript letters differ significantly ($P < 0.05$).

This may be due to the thermal deactivation of phenolic compound during baking process and formation of indigestible complexes with SFP protein and lipid, which agrees with previous studies such as the enrichment of bread with quinoa leaves (Swieca et al., 2014). Phenolic compounds are susceptible to exposure to light, oxygen and heat which are normally present in food processing. The total antioxidant activities (TEAC) of 15 % SFP bread were significantly higher at than those of the control bread.

Similarly, Swieca et al., (2013) observed reduced phenolic content and the masking of antioxidant potential of enriched bread with onion skin. Reduction in phenolics could be attributed to phenolics present in the SFP that might have formed protein-phenolics or phenolic-lipid complex via hydrogen and /or hydrophilic interactions (Swieca et al., 2013).

10.3.6 Conclusion

Fortification of bread with SFP is an effective technique to improve the protein, essential amino acids and fatty acids composition of the final product. The addition of SFP significantly increased the protein and energy content of the bread. Thus, creating a bread with a higher nutritional value as measured by PDCAAS, BV and NI which make them potentially valuable to be used as source of protein. The results indicate that inclusion of SFP in bread may give acceptable volume, crumb and textural properties. SFP bread inhibits starch digestion by increasing the protein matrix around the starch granules thereby lowering the release of reducing sugars. The antioxidant capacity of the bread was significantly enhanced by SFP inclusion. These results indicate that SFP fortification might be a promising way to produce a product with maximum potential nutritional and health benefits.

Chapter 11

Effect of incorporation of fish oil and wheat gluten to wheat starch on physicochemical, structural and *in vitro* digestion properties of starch

This chapter is submitted for publication in “**Food Hydrocolloids**” Journal.

Ajay S. Desai., Margaret A. Brennan., Xin-An Zeng., & Charles S. Brennan. Effect of incorporation of fish oil and wheat gluten to wheat starch on physicochemical, structural and *in vitro* digestion properties of starch.

Abstract

The food matrix composition and its structure can have a positive impact on physicochemical and digestion properties. In this study, three component mixtures (TCMs) were prepared by rapid visco analyser (RVA) with wheat starch (WS), 15% of cod oil (CO), salmon oil (SO), coconut oil (CONT) and 10% wheat gluten (WG). The aim of this study was to investigate the effect of starch-lipid-protein (ternary) complexes on the physicochemical, structural and *in vitro* enzymatic properties of starch and mechanism involved between WS, CO, SO, CONT and WG. Results exhibited that the TCMs samples showed a decreased peak viscosity, an increased pasting temperature and decreased enthalpy changes as compared to WS and WG due to the amylose-lipid complex formation. SEM images showed the more embedded structure of starch granules in TCMs samples as compared to WS. The colour and textural indices of WS were found to be significantly decreased in TCMs samples. The findings of Raman and Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and ¹³C NMR techniques showed that the structural changes occurred in TCMs samples with more ordered structure of complex formation with starch-lipid and protein. Results displayed

that a significant decreased in starch digestibility in TCMs samples as compared to WS in order of CO>CONT>SO>WG>WS. We conclude that fatty acid present in the lipids formed a more complexed structure with amylose and inhibit the attack of digestive enzymes for hydrolysis. The findings of this study help to design food products with low digestibility and improved nutritional profiles.

11.1 Introduction

The food matrix of stable foods is generally composed of starch, lipid, and protein in different concentrations as well as other minor constituents. During food processing, the complex interaction of these micronutrients take place to affect the physicochemical (food texture, viscosity, colour) and nutritional (starch digestibility/ glycaemic response), structural and organoleptic properties of finished food products (Parada & Santos, 2016; Zheng et al., 2018). Consumption of highly digestible starchy food has been shown to lead to the increased risk of many chronic diseases such as diabetes and obesity and therefore, food containing slowly digestible starch and resistant starch have been recommended to prevent such types of disease. Starch digestion depends on its linear chain amylose, branched chain amylopectin content and complex interaction of starch with lipids and proteins in the food matrix (Annor et al., 2015). To improve the final quality of food product and processing, lipids are generally used to formulate many foods. Single helical binary complex formation of amylose with different types of lipids have been well studied (Farooq, Dhital, Li, Zhang, & Huang, 2018; Reddy, Choi, Lee, & Lim, 2018; Wang, Wang, Yu, & Wang, 2016; Zhang, Huang, Luo, & Fu, 2012) and resulted into reduction in swelling power and solubility of starch, influence the pasting properties, increased gelatinisation temperature and resistance

to hydrolysis of starch. Amylose-lipid complexes (ALC) in food product increases the dissociation temperature with increase in the number of double bonds in the hydrocarbon chains and this has been used to formulate the starchy food products with enhanced properties (Chen et al., 2018b). Wang, Wang, Yu, & Wang (2016) investigated the effect of different chain lengths and double bonds of fatty acids on thermal, pasting, swelling and enzymatic hydrolysis properties of starch. The result showed that all the lipids used in this study, form an amylose-lipid complex which inhibits the swelling, gelatinisation, pasting and starch hydrolysis rate. Starch-protein interactions in food products and their effects on rheological and digestibility properties were well studied (Akinwale, Shittu, Adebawale, Adewuyi, & Abass, 2017; Kumar, Brennan, Zheng, & Brennan, 2018; Li, Wang, Chen, Yu, & Feng, 2018; Liu et al., 2018b; Oñate Narciso & Brennan, 2018). Ternary complex interactions between starch, lipid, and protein have been confirmed by rapid visco analyser (RVA) and high performance size exclusion chromatography (HPSEC) studies (Shah et al., 2011). These complexes have a significant effect on rheological, functional and nutritional properties of the starch (Bhattarai, Dhital, & Gidley, 2016). Chen et al. (2017a) investigated the effect starch (corn)-lipid (corn oil)-protein (soy protein) interaction on the physiochemical and *in vitro* starch digestibility properties of the starch. They concluded that formation of ternary complex reduced the pasting temperature, enthalpy of amylose-lipid complex and *in vitro* starch digestibility. Also, Zheng et al. (2018) reported that the ternary complex (starch- β -lactoglobulin-fatty acid) formed a more ordered V type complex than those of binary (starch-fatty acid) complex. The study on the digestibility of starch-lipid-protein complexes is still not well understood. In this study, we prepared the three component mixture (TCMs) consist of wheat starch (WS), salmon oil (SO), cod oil (CO), coconut oil

(CONT) and wheat gluten (WG) with RVA. The objective of this study was to investigate the effects of oil and protein on physiochemical, rheological, structural and digestion properties of wheat starch in ternary component blends.

11.2 Materials and methods

11.2.1 Raw materials

Described in section 3.1

11.2.2 Wheat starch-fishoil-gluten gel preparation

Described in section 3.4.15

11.2.3 Complex Index (CI)

Described in section 3.4.16

11.2.4 Pasting properties

Described in section 3.4.15

11.2.5 Texture and colour characteristics

Described in section 3.4.9 and 3.4.13.4

11.2.6 *In vitro* starch digestibility

Described in section 3.4.5

11.2.7 Scanning electron microscopy (SEM)

Described in section 3.4.17

11.2.8 Fourier transform infrared (FT-IR) spectroscopy

Described in section 3.4.18

11.2.9 Laser confocal micro-Raman (LCM-Raman) spectroscopy

Described in section 3.4.19

11.2.10 Differential scanning calorimeter (DSC)

Described in section 3.4.20

11.2.11 ¹³C nuclear magnetic response (NMR) spectroscopy

Described in section 3.4.21

11.2.12 Statistical analysis

Described in section 3.4.22

11.3 Result and discussion

11.3.1 Pasting, texture and colour characteristics

The pasting profiles of WS, WS-WG and TCMs samples analysed by RVA are illustrated in Table 11.1. The peak viscosities of TCMs samples were significantly lower ($p < 0.05$) as compared to the WS sample. In TCMs samples, starch granules could not swell completely due to the formation of amylose-lipid complexes and gluten acts as a physical barrier during the gelatinisation process. Also, starch and protein are competing each other for water to hydrate and swell. A similar finding has been made by (Chen et al., 2018b; Zheng et al., 2018) who reported that peak viscosity of three complex mixtures (maize starch-maize oil-zein protein) and (maize starch- β -lactoglobulin-fatty acids) were decreased compared to those of maize starch respectively. During the heating process, protein gets denatured to form a protein network on the surface of starch granules resulting in retard the process of starch swelling (Ai et al., 2013). The above results were consistent with CL, DSC, FTIR, and XRD result that lipid contain shorter carbon chain length form more complex structure. The pasting temperature of TCMs was increased as compared to WS. The pasting temperature increased from 88.56 °C to 92.48 °C for WS and TCMs samples. The increase in pasting temperatures are consistent with the inhibiting swelling of starch

Table 11.1 Pasting characteristics of wheat starch and its blend with 15% cod, salmon, coconut oil and 10% wheat gluten.

Samples	Peak Viscosity (cP)	Trough Viscosity (cP)	Break down Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Pasting Temperature (°C)	Complex index (%)
WS	2848 ±12.52 ^a	2265 ± 14.18 ^a	579 ±16.86 ^a	3355 ± 40.95 ^a	1056 ± 33.77 ^b	88.56 ± 0.93 ^b	
WS+WG	2059 ± 10.44 ^b	1434 ± 13.60 ^b	625 ± 11.53 ^b	2436 ± 11.93 ^c	1002 ±14.01 ^b	89.38 ± 0.41 ^b	14.39 ± 0.05 ^d
WS+CO+WG	1592 ± 8.44 ^c	1149 ± 18.74 ^c	442 ± 18.44 ^c	1904 ± 9.01 ^d	755 ± 17.53 ^d	90.21 ± 0.49 ^b	17.13 ± 0.08 ^c
WS+SO+WG	1591 ± 5.13 ^c	1056 ± 8.18 ^d	535 ± 12.74 ^{ab}	1905 ± 9.59 ^d	849 ± 16.37 ^c	89.63 ± 0.02 ^b	20.84 ± 0.1 ^b
WS+CONT+WG	1087 ± 4.01 ^d	759 ± 9.13 ^f	327 ± 11.77 ^d	1338 ± 13.45 ^e	579 ± 8.01 ^e	92.48 ± 0.37 ^a	24.23 ± 0.7 ^a

WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten;

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation.

Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.¹¹⁵

granule by the interaction between starch and lipid/protein. Wang et al. (2016) reported that the pasting temperatures of complex mixtures (wheat starch-fatty acids) were increased as compared to wheat starch alone. The gel hardness of WS, WS-WG and TCMs gels were determined by texture analysis and presented in Table 11.2.

Table 11.2 Texture and colour characteristics of wheat starch gel and its blend with 15% cod, salmon, coconut oil and 10% wheat gluten.

Samples	Measurements				
	L^*	a^*	b^*	ΔE	Hardness (g)
WS	78.56 ± 0.25^e	-9.17 ± 0.05^a	17.96 ± 0.18^e		45.30 ± 0.74^a
WS+WG	81.04 ± 0.22^d	-9.83 ± 0.04^b	19.81 ± 0.04^d	3.25 ± 0.18^d	26.02 ± 0.51^b
WS+CO+WG	84.02 ± 0.47^b	-9.94 ± 0.27^{bc}	21.03 ± 0.50^c	6.53 ± 0.39^b	21.21 ± 0.39^c
WS+SO+WG	87.01 ± 0.39^a	-10.16 ± 0.13^c	22.93 ± 0.26^b	9.75 ± 0.45^a	19.19 ± 0.27^d
WS+CONT+WG	83.22 ± 0.26^c	-9.94 ± 0.05^{bc}	20.98 ± 0.16^c	5.67 ± 0.44^c	21.18 ± 0.68^c

WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: coconut oil; WG: wheat gluten. Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation ($n=6$). Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

In this study, gel hardness of WS (42.30 N) and TCMs (19.19N) were observed. The gel hardness in TCMs samples decreased significantly ($p < 0.05$) as compared to WS. These results suggest that, during the heating process, amylose-lipid complexes (ALC) were formed in TCMs samples and as a result their gel forming ability led to a softer gel which was more easier to penetrate as compared to WS. This ALC formation may prevent the interaction between starch molecules to form double helices structure and strong gel network (Ai et al., 2013). Similar findings have been described by Yu, Wang, Chen, Li, & Wang (2018), who found that wheat starch gels with the inclusion of stearic acid and sodium alginate showed lower gel harness than those of wheat starch. For visual appreance colour of WS and TCMs gel were measured and presented in Table 11.2. There has been a significant difference ($p < 0.05$) were observed between WS and TCMs gel samples in terms of L^* (Lightness), a^* (redness) and b^* (yellowness) values. The

increased in the L^* , a^* and b^* values may be due to release of the colour compound from TCMs during heating process as cod, salmon oil and wheat gluten in its ingredients form reflect yellow and light brown colour respectively. Colour differential index (ΔE) was found to be more noticeable with TCMs sample than those of WS. Overall, colour of gel with TCMs samples appeared to the yellowish-brown as compared to WS gel alone. In another study, (Kumar, Brennan, Mason, Zheng, & Brennan, 2017) reported that inclusion of whey protein and skim milk powder to oat starch showed an increase in b^* value of gel parameter while there is difference were observed in L^* and a^* values.

11.3.2 Thermal properties and complex index (CI)

The thermal transition temperatures (T_o , T_p , T_c) and gelatinisation enthalpy change (ΔH) of WS and TCMs gel samples are shown in Table 11.3.

Table 11.3 Thermal properties of wheat starch and in combination with (15%) cod oil, salmon oil, coconut oil and (10%) wheat gluten.

Samples	Peak			
	$T_o(^{\circ}\text{C})$	$T_p(^{\circ}\text{C})$	$T_c(^{\circ}\text{C})$	ΔH (J/g starch)
WS	57.43 ± 0.30^b	62.36 ± 0.25^b	67.13 ± 0.15^b	3.69 ± 0.00^a
WS+WG	58.10 ± 0.10^a	63.61 ± 0.20^a	68.21 ± 0.18^a	2.20 ± 0.00^b
WS+CO+WG	58.10 ± 0.15^a	63.53 ± 0.16^a	68.36 ± 0.26^a	1.82 ± 0.00^c
WS+SO+WG	58.11 ± 0.10^a	63.16 ± 0.15^a	68.33 ± 0.20^a	2.05 ± 0.04^b
WS+CONT+WG	58.20 ± 0.15^a	63.46 ± 0.15^a	69.33 ± 0.20^a	2.43 ± 0.00^b

WS: wheat starch; CO: cod oil; SO: salmon oil; SA: CONT: coconut oil; WG: wheat gluten. Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

WS exhibited gelatinisation thermal transition temperatures in the range of 57.43-67.13 $^{\circ}\text{C}$ and enthalpy change of 3.69 J/g. WS-WG mixture showed 58.10-68.21 $^{\circ}\text{C}$ transition temperatures and 2.2 J/g enthalpy. The increase in the onset temperature and decreased in enthalpy have been observed among the WS and WS-WG mixture gel

suggest that gelatinisation of starch was affected. This may be due to the presence of protein (gluten) restrict the supply of water to gelatinise the starch molecules. Similar findings were also reported for starch- β -lactoglobulin complexes by (Wang et al., 2017). The TCMs showed thermal transition temperatures in the range of 58.10-69.33 °C and enthalpy change of 1.82-2.43 J/g. Compared to WS, TCMs exhibited an increase in the T_o , T_p , T_c a decrease in the enthalpy change (ΔH) and an increased in the gelatinisation temperature range ($T_c - T_o$). The increase in the gelatinisation temperature of TCMs can be attributed to the formation of amylose-lipid complex (Yu, Wang, Chen, Li, & Wang, 2018). This could be hydrophobic characteristics of lipids in the TCMs have an ability to restrict the water supply for starch gelatinisation. Another reason may be due to the electrostatic interaction between an amino acid group of gluten and the carboxylic group of fatty acids which bring TCMs together (Wang et al., 2016). The decrease in enthalpy changes in TCMs samples due to the addition of lipid and protein with starch which alters the junction forces between the amorphous region and crystallites (Yu et al., 2018). Wang et al. (2017) also found that the enthalpy value of three components blends (maize- β -lactoglobulin-lauric acid) was significantly reduced as compared to maize starch alone. They reported that due to the presence of lauric acid gelatinisation of starch was inhibited and also there was the formation of type I complex of maize starch-lauric acid which alters the starch gelatinisation properties.

The complex index (CI) of WS, WS-WG and TCMs samples are shown in Table 11.1. The CI represents the complex formation between starch-lipid-protein and is work on the principle of the affinity of starch molecules towards iodine (Annor et al., 2015). In this study, CI of WS-WG and TCMs were found to be the ranges of 14.39% and 17.13 % - 24.23% respectively. In this study, the CI index is significantly ($p < 0.05$) increased with

decreasing carbon chain length of fatty acids presents in the TCMs and gluten. The result showed that saturated fatty acids can form complex more easily than unsaturated fatty acids. However, during gelatinisation, short chain fatty acids easily interact with amylose to form more complex structure and higher CI. Similar findings were reported by Oyeyinka, Singh, Venter, & Amonsou, (2017) and Wang et al., (2016).

11.3.3 Laser confocal micro-raman (LCM-Raman) spectra of ternary complexes and its effect on short range ordered structure

To evaluate the complex formation and structures of ternary complexes in TCMs, their short range molecular order was analysed by Raman spectroscopy. The spectra of WS, WS-WG and TCMs samples obtained from Raman are presented in Figure 11.1 and the full width height maximum (fwhm) of the band at 480 cm^{-1} are shown in Table 11.4.

Table 11.4 FWHM of the band at 480 cm^{-1} band and at $20\text{ (}2\theta\text{)}$ angle determined by Raman and XRD respectively of wheat starch and in combination with 15% cod oil, salmon oil, coconut oil and (10%) wheat gluten.

Samples	FWHM at 480 cm^{-1}	FWHM at $20\text{ (}2\theta\text{)}$ angle
WS	10.12 ± 0.01^a	13.15 ± 0.20^a
WS+WG	11.18 ± 0.02^a	12.45 ± 0.18^a
WS+CO+WG	5.72 ± 0.01^b	10.57 ± 0.14^b
WS+SO+WG	5.62 ± 0.01^b	10.88 ± 0.17^b
WS+CONT+WG	6.03 ± 0.02^c	9.97 ± 0.11^c

WS: wheat starch; CO: cod oil; SO: salmon oil; SA: CONT: coconut oil; WG: wheat gluten. Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

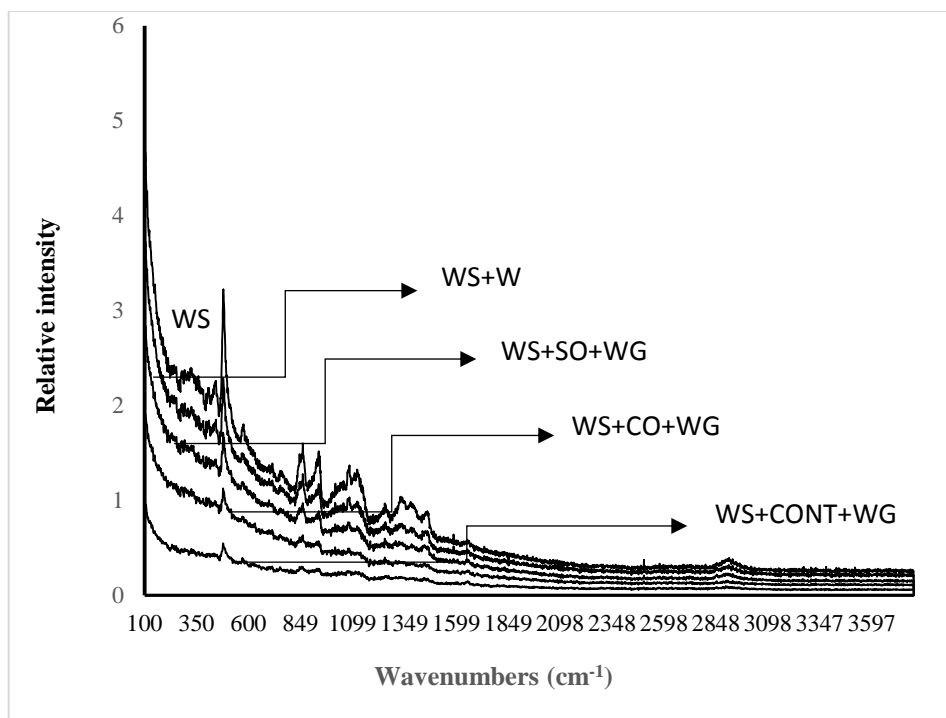


Figure 11.1 LCM-Raman spectroscopy of WS, WS-WG and TCMs samples. Sample code: Wheat starch (WS), SO: salmon oil; CONT: Coconut oil; wheat gluten (WG).

The fwhm at 480 cm^{-1} can be used to describe the order degree of starch structure. The samples show smaller fwhm value and stronger absorption intensity at this wavelength revealing highly order starch structure (Zheng et al., 2018). The LCM-Raman spectra of WS, WS-WG, and TCMs showed the strongest band at 480, 865, 1060, 1350 and 2900 cm^{-1} . The fwhm value of WS, WS-WG and TCMs samples ranged from 10.12, 11.18 and 5.62-6.03. The TCMs sample had the significantly ($p < 0.05$) smallest fwhm followed by WS and WS-WG. This results indicated that inclusion of different oils (lipids) with wheat gluten (protein) to wheat starch form more complex V-type crystalline structure, consistent with results obtained from FTIR, XRD, DSC, and NMR. Similarly, Wang et al. (2017) reported that three blends complex maize starch (MS)- β -lactoglobulin(β LG)-lauric acid(LA) form greater short range molecular order with lower fwhm value as compared to MS and MS- β LG.

11.3.4 Effect of different oil sources on FTIR spectra of ternary complexes

The FTIR spectra of WS and TCMs obtained from RVA pasting are depicted in Figure 11.2. Compared to WS, TCMs exhibited more pronounced absorption at 1747 and 2853 cm^{-1} . The more distinct absorption peak at 2853 cm^{-1} have been observed in TCMs samples compared WS-WG, whereas no peak detected in WS to this wavelength (Figure 11.2).

Figure 11.2 FTIR spectra of WS, WS-WG and TCMs samples. Sample code: WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten

The intensity of peak increase can be attributed to increasing carbon chain length and saturated fatty acids (WS+CONT+WG> WS+CO+WG>WS+SO+WG>WS+WG). Another reason may be an interaction between the hydroxyl group and a methylene group of fatty acids complex (Wang et al., 2017). The intense peak at 1747 cm^{-1} represents the carbonyl group of fatty acids present the TCMs and increased with saturated fatty acids. This may be due to the formation of a starch-lipid complex by the esterification between WS with added different oils in TCMs. The amide region I (1600-1700) and II (1500-1600)

of FTIR spectra have the most important bands of the polymer chain with protein backbone. The amide I is corresponding with C=O stretching and amide II mainly described from N-H bending and C-N stretching (Chen et al., 2018a). The TCMs and WS-WG complexes had a characteristic FTIR absorption band at about 1549 cm^{-1} as compared to WS, which is connected to the deformation vibration of amino group and absorption of amide II in wheat gluten. Similarly, Chen et al. (2018b) found that maize starch (MS)-maize oil (MO)-zein protein (ZP) blends form V type starch-lipid-protein complex with different band wavelength. In contrast to this, Wang et al. (2017) who reported that ternary complexes (starch- β -lactoglobulin-fatty acid) were not shown absorbance band at 1710 cm^{-1} and 2846 cm^{-1} in FTIR spectra due to the weakening of carbonyl group of lauric acid.

11.3.5 X-ray diffraction of ternary complexes

The X-ray diffraction pattern and full width height maximum (FWHM) at 20° (2θ) of WS, WS-WG and TCMs samples obtained after RVA pasting are shown in Figure 11.3 and Table 11.4. WS and WS-WG showed the A-type crystalline structure with strong reflections peaks at 2θ values of 17.5° and 20° . The TCMs exhibited V type patterns as compared to WS and WS-WG at 20° representing the formation of amylose-lipid complexes (Chen et al., 2018b).

Figure 11.3 XRD spectra of WS, WS-WG and TCMs samples. Sample code: WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten.

This result is consistent with fwhm value obtained with TCMs samples gave much lower fwhm values (10.57- 9.97) as compared to WS (13.15) and WS-WG (12.45). The lower fwhm value observed in TCMs which indicated that stronger amylose lipid complexes have been formed Wang et al. (2017). Another reason may be the presence of saturated fatty acids with increasing carbon chain length in TCMs as compared to WS and WS-WG. This finding indicated that complex formation between starch-lipid-protein followed the order TCMs>WS-WG>WS. This is in general agreement with the CI and DSC results that short chain fatty acids form complex and exhibited enthalpy changes. Similarly, Chen et al. (2018b) and Wang et al. (2017) reported that ternary blends of MS-MO-ZP and MS-LA- β LG formed V type amylose-lipid complexes at θ values of 20° and 19.8°

respectively while MS and MS-BLG showed no diffraction peaks indicated that no crystalline structure had been formed.

11.3.6 *In vitro* starch digestibility

The food matrix are composed of starch, protein and lipid constituents which play a vital role in the release of an amount of reducing sugar in the small intestine during the digestion process. In this study, the effect of different lipid sources cod oil (CO), salmon oil (SO) and coconut oil (CONT) and wheat gluten (WG) on *in vitro* starch digestibility properties of wheat starch were examined. Figure 11.4 showed the amount of reducing sugar released over 120 min during the *in vitro* digestion of TCMs, WS and WG samples.

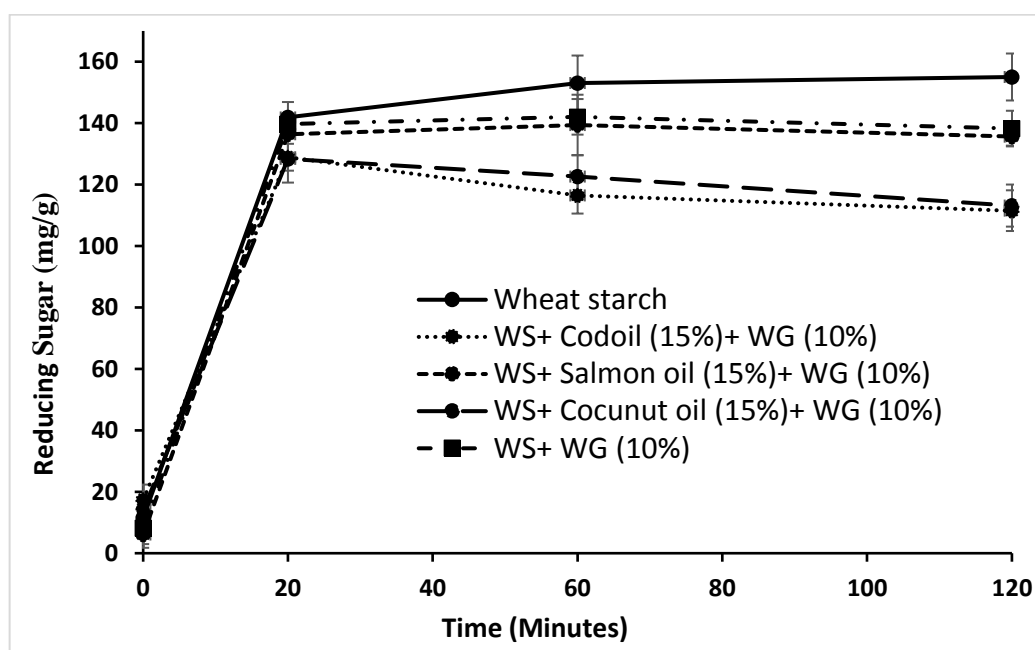


Figure 11.4 Amount of reducing sugar released during *in vitro* digestion for wheat starch and replacement of wheat starch with 15 % cod oil, salmon oil, coconut oil and 10 % wheat gluten.

It can be observed that during the first 20 min digestion, all samples illustrated a rapid increase in the starch degradation and with increase digestion time (20-120 min), the starch digest slowly (Figure 11.4). Compared to the WS and WG, TCMs samples

exhibited a significant ($p < 0.05$) decrease in the amount of reducing sugar at each time point. These findings were similar with those reported by Zheng et al. (2018) who showed that ternary complexes (MS-LA- β LG) were more resistance to degrade the amylase due to the more structural order complex formed between maize starch and lauric acid. It also demonstrated that the presence of β LG react with α -amylase and decrease the starch digestibility. The amylose-lipid complex formation ability in food matrix in mainly depend on lipid type used and its chain length (Ren et al., 2016). The TCMs sample contains short chain saturated and long chain unsaturated fatty acids compared to the WS and WG and due to this, more V-amylose complex with lipids formed which could inhibit the activity of α -amylase which is responsible for starch hydrolysis (Chen et al., 2018b). These results are consistent with the FTIR (Figure 11.2) and XRD (Figure 11.3) results. Ye et al. (2018) and Annor, Marcone, Bertoft, & Seetharaman (2013) suggested that lipid and protein encapsulated starch granular matrix in rice flour and millet was important for its glycaemic index and revealed that presence of lipid and protein can form V-complex structure and restrict the swelling of starch granules which results in the decrease in the starch digestibility. Zhang, Maladen, Campanella, & Hamaker (2010) also reported that formation of a stable ternary complex in the food matrix depends on negatively charged lipid carboxyl group that helps to form the stronger bond between amylose and positively charged protein molecules.

11.3.7 Ternary complex detection using ^{13}C nuclear magnetic response (NMR) spectroscopy

^{13}C NMR techniques used to describe the starch molecular structure at short distance scale. The obtained spectra from the sample were categorised into amorphous, single

and double helical components. The signals from different peaks position were identified as C1 (120-140 ppm), C2, C3 and C5 (71-80 ppm), C4 (95-105 ppm) and C (60-65 ppm) (Chen et al., 2017a).

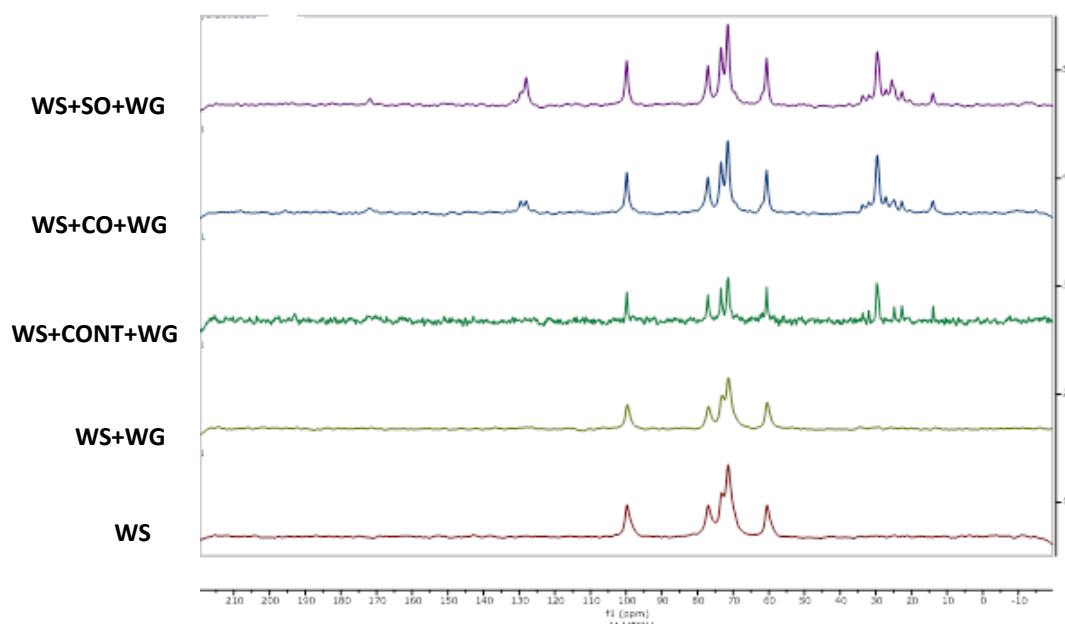


Figure 11.5 NMR spectra of WS, WS-WG and TCMs samples. Sample code: WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten

The change in torsion angles of the glycosidic bonds and structure of amylose molecule from a random coil to helix may be caused due to the complex formation with added lipids. In NMR spectrum, the distribution of electron patterns to the carbon 1 and carbon 4 bonds were affected by the change in torsion angles and resulted in downfield changes in the chemical shifts of carbon 1 and 4 bonds of a glucose unit. These two carbon sites exhibited more chemical shift changes between amorphous and v type complex structure indicating a structural alteration in the wheat starch molecules (Tan, Flanagan, Halley, Whittaker, & Gidley, 2007).

Table 11.5 ¹³C- chemical shifts of wheat starch and its blend with 15% cod, salmon, coconut oil and 10% wheat gluten.

Samples	Chemical shift (ppm)					
	C1	C2	C3	C4	C5	C6
WS+WG	0.00	-0.0004	-0.0009	-0.0006	-0.0009	-0.0007
WS+SO+WG	0.003	0.002	0.002	0.005	0.001	0.001
WS+CO+WG	0.004	0.002	0.002	0.005	0.001	0.001
WS+CONT+WG	0.003	0.002	0.001	0.005	0.001	0.001

WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: coconut oil; WG: wheat gluten. Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

In this study, ¹³C chemical shifts of carbon 1 and 4 bond of amylose with cod oil (CO), salmon oil (SO) and coconut oil (CONT) and wheat gluten (WG) samples showed that fatty acid present in the CO, SO and CONT formed more complex helical structure as compared to WG (Figure 11.5 and Table 11.5). This result is consistent with XRD and FTIR finding (Figure 11.2 and 11.3). From this finding, it can be concluded that saturated and polyunsaturated fatty acid present in the TCMs formed complexes with amylose and inhibit the swelling of starch granules which decreased the starch digestibility viscosity and texture of the TCMs samples compared to WS and WS+WG. Similar findings were reported by Ai, Hasjim, & Jane (2013) who demonstrated that inclusion of different lipid sources to the starch matrix decrease the enzymatic hydrolysis and viscosity property due to the fatty acid and amylose complex formation.

11.3.8 Morphological properties

The morphological characteristics of WS, WS+WG and TCMs samples were studied by scanning electron microscopy (SEM) (Figure 11.6).

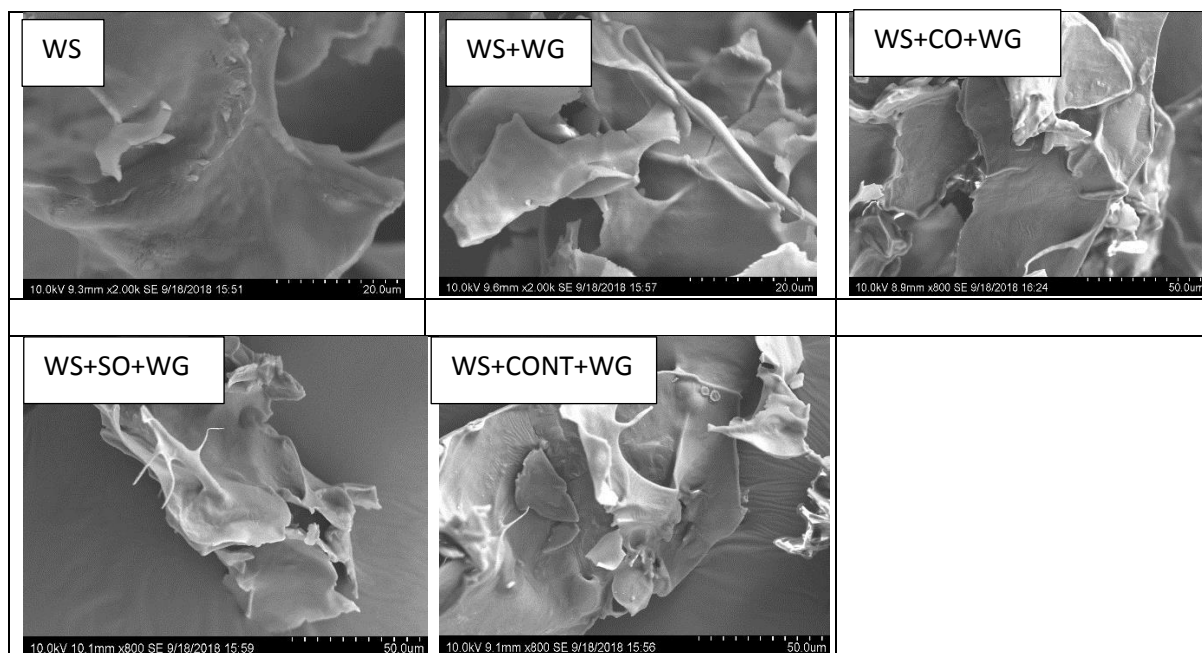


Figure 11.6 Scanning electron microscopic images (800 x magnification) of wheat starch (WS) and its blends with 15% salmon oil (SO), cod oil (CO), coconut oil (CONT) and 10% wheat gluten (WG)

The result showed that WS exhibited a smooth surface and TCMs samples were partially swollen and gelatinise after thermal treatments due to the interaction of amylose- lipid –gluten. It can be seen that fatty acids and protein molecules of TCMs samples were embedded in the starch granule as compared to WS and WS+WG. This can be attributed to the formation of an amylose-lipid complex in TCMs samples and also hinders of protein molecules into the starch matrix (Figure 11.6). Similar observations were made by Chen et al., (2017a, 2018b) in the ternary complex of corn oil-soy protein-corn starch and maize starch-maize oil-zein protein. The results showed that corn and maize starch granules were embedded in the soy and zein protein and corn and maize oil matrix respectively and revealed that fatty acid and protein present in the starch matrix accelerate the formation of a ternary complex.

11.4 Conclusion

This study exhibited a significant effect on physicochemical, texture and *in vitro* digestion properties of the starch with the inclusion of different lipid sources (SO, CO and CONT) and/or wheat gluten and interaction between them. The TCMs samples indicated the more embedded structure of the granules' compared to other samples. The addition of lipid and protein to wheat starch demonstrated a decrease in the enthalpy change. Result also showed that, with the addition of SO, CO and CONT and gluten, WS displayed a decreased peak viscosity and an increased in pasting temperature, resulting from the less starch gelatinisation due to the ternary complex formation. Also, the texture and colour properties of WS were affected by the addition of lipid and protein sources. The inclusion of lipid and protein to WS exhibited the V type X-ray pattern, more ordered structure FTIR peak and chemical shift of carbon 1 and carbon 4 bond, indicated the formation of the ternary complex (starch-lipid-protein). TCMs contain short chain fatty acids and polyunsaturated fatty acids exhibited a decrease in starch digestibility as compared to WS and WS+WG. Lipid plays more influence on enzymatic hydrolysis of starch compared to gluten. This study delivers a better knowledge for the formation of a ternary complex of different lipid sources with different fatty acid composition and gluten. These findings will help to the food industry to design food product with control enzymatic hydrolysis of starch.

Chapter 12

Effect of incorporation of fish oil to wheat starch on pasting, textural, *in vitro* starch digestibility and structural properties of the starch

This chapter is submitted for publication in “**International Journal of Biological Macromolecules**” Journal.

Ajay S. Desai., Margaret A. Brennan., Xin-An Zeng., & Charles S. Brennan. Effect of incorporation of fish oil and wheat gluten to wheat starch on physiochemical, structural and *in vitro* digestion properties of starch.

Abstract

This study aimed to examine the effect of inclusion salmon oil (SO), cod oil (CO), coconut oil (CONT) and wheat gluten (WG) to wheat starch (WS) on pasting, textural and *in-vitro* starch digestibility properties. The binary component blends (BCBs) sample showed better complex index (CI) as compared to WS and WS-WG and decreased with increasing carbon chain length. The inclusion of lipids to WS significantly reduced pasting and textural characteristics than that of WS alone. In BCBs samples V type amylose-lipid complexes peak at $20^{\circ} 2\theta$ and greater short range molecular order were formed with fatty acids producing more crystalline structure. Similar observation was noted by FTIR, Raman, and NMR spectroscopy. Differential scanning calorimetry (DSC) results suggested that decreased in the gelatinisation temperature and enthalpy after addition of different oil sources to WS. The amount of reducing sugar released during starch digestion appears to be dependent on starch hydrolysis. In this study, due to amylose-lipid complex formation, *in vitro* glycaemic response decreased in BCBs samples followed by WS-WG with the ability to act as a barrier to suppress the activity of

digestive enzymes to hydrolyse the starch molecules. This altered characteristics of WS might help to formulate the foods to overcome the digestibility concern in human.

12.1 Introduction

In the cereal food products, most important polysaccharides available is wheat starch that plays a vital role to alter the food characteristics such as viscosity, texture, colour, and digestibility of finished product during processing. The food attributes quality obtained from pasting and gelatinisation of starch can be affected by the inclusion of protein and lipid constituents (Wang et al., 2016). To improve the processing condition and quality of finished products, lipids are commonly used to modify the functional properties of starchy foods. Starch consists of two major polymers i.e. amylose (linear chain) and amylopectin (branched chain). Out of which, linear chain amylose can interact with added lipids to form a single helical inclusion complex (Farooq et al., 2018). It is well defined that such complex formation exhibited decreased pasting and textural (Oyeyinka et al., 2017), glycaemic index (Ai et al., 2013; Kawai, Takato, Sasaki, & Kajiwarra, 2012) and thermal properties (Liu, Wang, Kang, Cui, & Yu, 2018a) of starch. The formation and stability of amylose-lipid complex in starch paste mainly depend upon lipid chain length, its saturation, and presence of hydrophobic force between amylose helical cavity and lipids source (Gunenc, Kong, Elias, & Ziegler, 2018). Wang et al., (2016) reported that complex formation ability in wheat starch with lauric, myristic and palmitic acid decreased with increasing carbon chain length and it inhibits the swelling and starch hydrolysis of starch. So far, the effect of saturated fatty acids on rheological and digestibility properties of starch has been widely studied (Liu et al., 2018a; Oyeyinka et al., 2017; Reddy et al., 2018; Wang, Liu, Cui, Kang, & Yu, 2019) (Li et

al., 2019), the pasting, textural and digestion properties of the wheat starch complex with long chain polyunsaturated fatty acids (LCPUFA) are not well studied. Starch based snack foods from corn, rice, and wheat are widely consumed and represent a key part of the global diet. However, the composition and processing of these snack foods mean that they possess high levels of rapidly digestible starch which in turn raises nutritional and health issues such as obesity and cardiovascular disease and leads to suggestions that high starch food should be fortified with fish lipid to reduce the glycaemic impact of such foods and provide a balanced nutritional profile (Thachil, Chouksey, & Gudipati, 2014). Fish oil is a rich source of LC ω -3PUFA including α -linoleic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which contains 18, 20, 22 and 3, 5, 6 carbon atom and double bonds respectively. These fatty acids are abundantly available naturally in the salmon and cod fish. The international Society of the Study of Fatty Acids and Lipids (ISSFAL) recommended intake of 0.65 g of ω -3 fatty acids (EPA+DHA) per day. It has a positive impact on human health to prevent chronic diseases such as cardiovascular, inflammation, cancer and diabetes (Saini & Keum, 2018). Fatty acids with long hydrocarbons chain and double bond tend to form more ordered amylose-lipid complex that inhibits the enzymatic hydrolysis of starch (Ai et al., 2013). The aim of this study was to investigate the effects of different lipid sources on the complex index, pasting, textural, thermal and *in vitro* digestibility of wheat starch. The formation of amylose and fatty acids complex and its effect on technological and functional properties of starch were examined by complexing index, viscosity, X-ray diffraction, LCM-Raman, FTIR spectroscopy, and ^{13}C NMR.

12.2 Materials and methods

12.2.1 Raw materials

Described in section 3.1

12.2.2 Wheat starch-fishoil (starch-lipid) gel preparation

Described in section 3.1

12.2.3 Complex Index (CI)

Described in section 3.4.15

12.2.4 Pasting properties

Described in section 3.4.16

12.2.5 Texture and colure characteristics

Described in section 3.4.9 and 3.4.13.4

12.2.6 *In vitro* starch digestibility

Described in section 3.4.5

12.2.7 Scanning electron microscopy (SEM)

Described in section 3.4.17

12.2.8 Fourier transform infrared (FT-IR) spectroscopy

Described in section 3.4.18

12.2.9 Laser confocal micro-Raman (LCM-Raman) spectroscopy

Described in section 3.4.19

12.2.10 Differential scanning calorimeter (DSC)

Described in section 3.4.20

12.2.11 ¹³C nuclear magnetic resonance (NMR) spectroscopy

Described in section 3.4.21

12.2.12 Statistical analysis

Described in section 3.4.22

12.3 Results and discussion

12.3.1 Pasting, textural and colour characteristics

The pasting characteristics of all samples analysed by RVA are depicted in Table 12.1. Peak, trough and breakdown viscosity of WG and BCBs samples were significantly lower as compared to WS alone. This could be attributed to the restriction of the supply of water for starch granules swelling due to the inclusion of lipids as had been noted previously (Okumus, Tacer-Caba, Kahraman, & Nilufer-Erdil, 2018; Oyeyinka et al., 2017). In addition the inclusion of different lipid sources to WS increased set back viscosity. Another reason for the decrease in peak viscosity of BCBs and WG may be due to the formation of amylose and lipid/protein complexes. The pasting temperatures showed a decreased trend except for WG as compared to WS. Similar observations were made by Ai et al., (2013) who reported that inclusion of corn oil to corn starch exhibited a decreased peak viscosity and pasting temperature. The gel hardness of WS, WG, and BCBs samples are showed in Table 12.2. The result showed that with the inclusion of lipids, the gel hardness decreased in WG and BCBs samples compared to that of WS alone. This result suggested that the addition of lipids make the gel softer and easy to penetrate as compared to WS alone. The reason may be due to the addition of lipids with SFA and LCPUFA form the inclusion complex with amylose and these complexes inhibit the interaction between the starch molecules preventing the formation continues gel matrix (Oyeyinka et al., 2017; Yu et al., 2018). The colour characteristics *a* (lightness), *b* (yellowness) and *L* (lightness) of WS, WG, and BCBs samples are showed in Table 12.2. The BCBs and WG samples were exhibited higher lightness and yellowish

value as compared to WS alone as revealed by comparatively high L^* values (80.72 - 84.59) and b^* value (19.11-19.65) and a^* value (9.80-10.05). The BCBs samples look whiter with higher L values than WS and WG (Table 12.2), however, the WG and BCBs samples were redder than WS alone. The darker colour of BCBs and WG samples may be attributed to the presence of long chain polyunsaturated fatty acids and pigments in the gluten (Yildiz et al., 2018).

Table 12.1 Pasting characteristics of wheat starch and its blend with 15% cod, salmon, coconut oil and wheat gluten.

Samples	Peak Viscosity (cP)	Trough Viscosity (cP)	Break down Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Pasting Temperature (°C)	Complex index (%)
WS	2848 ±12.52 ^a	2265 ± 14.18 ^a	579.66 ±16.86 ^b	3355.11 ± 40.95 ^a	1056 ± 33.77 ^c	88.56 ± 0.93 ^{ab}	
WS+WG	2059 ± 10.44 ^e	1434 ± 13.60 ^e	625 ± 11.53 ^b	2436.33 ± 11.93 ^c	1002 ±14.01 ^d	89.38 ± 0.41 ^a	14.39 ± 0.05 ^c
WS+CO	2631 ± 9.07 ^c	2159± 5.01 ^b	462 ± 4.16 ^c	3217.33 ± 7.76 ^b	1049 ± 6.92 ^{cd}	88.54 ± 0.36 ^{ab}	18.81 ± 0.04 ^b
WS+SO	2667 ± 3.21 ^b	1869 ± 9.01 ^d	777 ± 7.63 ^a	3210.66 ± 15.01 ^b	1360 ± 11.71 ^a	87.33 ± 0.05 ^{bc}	18.14 ± 0.16 ^b
WS+CONT	2541 ± 6.65 ^d	2085 ± 7.63 ^c	437 ± 5.68 ^c	3310.33 ± 9.50 ^b	1210 ± 4.04 ^b	87.19 ± 0.07 ^c	21.13 ± 0.09 ^a

WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: Coconut oil; WG: wheat gluten;

Results in the table represent the mean of triplicate measurements. Mean ± standard deviation.

Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

Table 12.2 Texture and colour characteristics of wheat starch gel and its blend with 15% cod, salmon, coconut oil and wheat gluten.

Samples	Measurements				
	L^*	a^*	b^*	ΔE	Hardness (g)
WS	78.56 ± 0.30^c	9.19 ± 0.02^a	18.16 ± 0.11^e		31.15 ± 0.81^a
WS+WG	80.72 ± 0.22^a	9.89 ± 0.03^b	19.11 ± 0.12^b	4.11 ± 0.04^d	30.12 ± 0.14^a
WS+CO	86.33 ± 0.30^c	9.81 ± 0.067^b	22.2 ± 0.09^c	8.23 ± 0.05^b	27.84 ± 0.52^b
WS+SO	87.86 ± 0.44^b	10.05 ± 0.043^b	23.36 ± 0.21^d	10.04 ± 0.11^a	27.23 ± 0.28^b
WS+CONT	84.59 ± 0.43^d	9.80 ± 0.01^a	19.65 ± 0.32^b	6.41 ± 0.32^c	30.52 ± 0.30^a

WS: wheat starch; CO: cod oil; SO: salmon oil; CONT: coconut oil; WG: wheat gluten. Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation ($n=6$). Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

12.3.2 Complex index (CI)

The complex index (CI) is based on a starch-iodine interaction principle and gives an indication of starch-lipid complex formation. It depends on the carbon chain length and unsaturation of fatty acids (Kawai et al., 2012). The CI of WG and BCBS samples ranged from 14.39% to 18.14-21.13% respectively (Table 12.1). The significantly higher CI was observed in the sample with coconut, salmon and cod oil as compared to WG sample. This may be attributed to the BCBS samples containing saturated and polyunsaturated fatty acids with lower carbon atoms and high double bonds respectively (Kawai et al., 2012). This result indicated that short chain fatty acid with lower carbon atoms form complexes more easily than long chain polyunsaturated fatty acids. Similar observation was made by (Kawai et al., 2012) who reported that starch mixed with linoleic acid form higher complex index and suggested that CI increased with increased the double bond in fatty acids. Also, in another study, reported that lipids with shorter chain fatty acids were reported to be better dispersed in the gelatinised starch and interact easily with

leached amylose molecules resulted into more complexes and higher CI (Wang et al., 2016).

12.3.3 Thermal properties

The gelatinisation transition temperatures (T_o , T_p and T_c) and enthalpy (ΔH) of WS, WG, and BCBs samples were depicted in Table 12.3.

Table 12.3 Thermal properties of wheat starch and in combination with (15%) cod oil, salmon oil, coconut oil and wheat gluten.

Samples	Peak			
	$T_o(^{\circ}\text{C})$	$T_p(^{\circ}\text{C})$	$T_c(^{\circ}\text{C})$	$\Delta H \text{ (J/g starch)}$
WS	57.43 ± 0.30^a	62.36 ± 0.25^a	67.13 ± 0.15^a	3.69 ± 0.00^a
WS+WG	58.10 ± 0.10^b	63.6 ± 0.21^b	68.21 ± 0.18^b	2.21 ± 0.02^c
WS+CO	57.13 ± 0.25^a	62.5 ± 0.20^a	67.46 ± 0.25^a	2.65 ± 0.03^b
WS+SO	57.33 ± 0.15^a	62.33 ± 0.20^a	67.11 ± 0.10^a	2.24 ± 0.04^c
WS+CONT	57.13 ± 0.25^a	62.5 ± 0.20^a	67.46 ± 0.25^a	2.66 ± 0.01^b

WS: wheat starch; CO: cod oil; SO: salmon oil; SA: CONT: coconut oil; WG: wheat gluten. Results in the table represent the mean of triplicate measurements. Mean \pm standard deviation ($n=3$). Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

WS exhibited gelatinisation transition temperatures in the range of 57.43-67.13 $^{\circ}\text{C}$ and enthalpy 3.69 J/g. The WS-WG sample showed significantly increased gelatinisation temperature (58.10-68.21) and decreased enthalpy (2.21 J/g) as compared to WS. These changes could be attributed to the presence of gluten restrict water supply to a starch molecule for swelling and gelatinisation. A similar observation was made by Wang et al., (2017) who reported that due to the presence of water soluble β lactoglobulin in the gel mixture of maize starch - β lactoglobulin decrease the water availability to starch. In BCBs samples gelatinisation temperature (57.33-67.46) did not show significance difference in transition temperature but there is decreased in the gelatinisation enthalpy (2.24-2.66 J/g) to that of WS. The reduction in the gelatinisation enthalpy of

BCBs samples may be ascribed to the limited water for starch swelling and gelatinisation (Yu et al., 2018). Such results are in accordance with the previous finding that addition of fatty acids to maize and wheat starch significantly decreased gelatinisation enthalpy value of starch (Wang et al., 2016). However, it is also possible that saturated fatty acids and LCPUFA present in the BCBs sample changes the hydrophobic force between and forms the complex with amylose and reduce the gelatinisation of starch.

12.3.4 Laser confocal micro-raman spectroscopy

The effect of the supply of different lipid sources to WS on complex formation and their short range molecular order structures were evaluated by Raman spectroscopy. It is reported that full width at half maximum (fwhm) at 480 /cm of Raman spectra can be used to indicate the order structure formation in starch, with lower fwhm value and high absorption intensity at this wavelength indicative of more firm complex formation (Wang et al., 2017). The Raman spectra and fwhm vale at 480 /cm of WS, WS-WG and BCBs samples were showed in Figure 12.1 and Table 12.4. WS showed more prominent Raman spectra at 480,600, 850, 1220 and 1410 /cm while BCBs samples were exhibited strongest bands, followed by WS-WG showed weak band to that of WS (Figure 12.1). The BCBs samples had the smallest fwhm value (10.38-10.67) followed by WS-WG (12.45), WS showed high fwhm value (13.15).

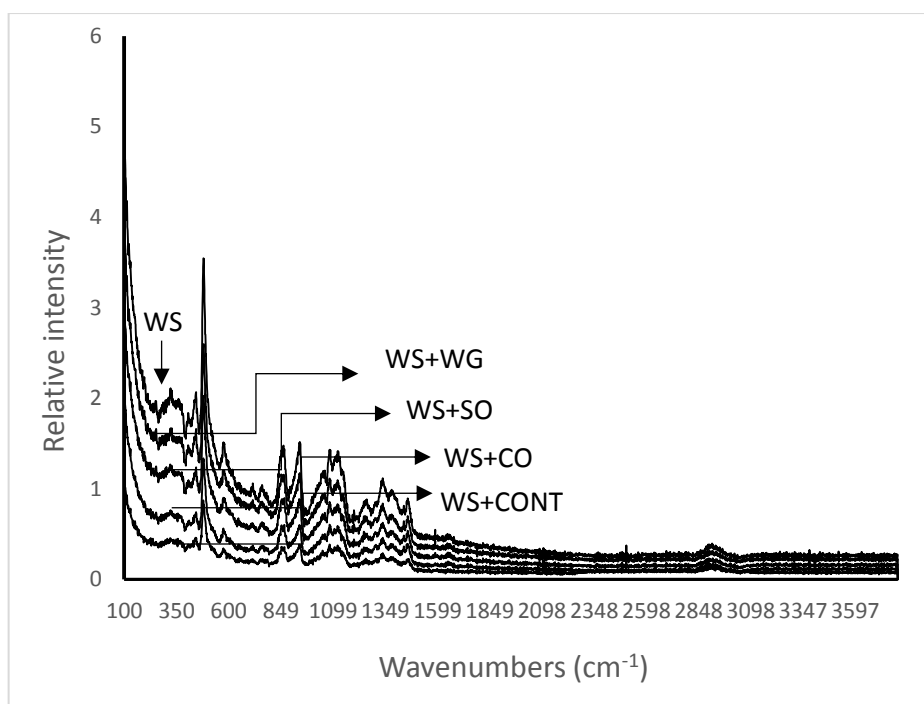


Figure 12.1 LCM-Raman spectroscopy of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).

Table 12.4 FWHM of the band at 480 cm⁻¹ band and at 20 (2θ) angle determined by Raman and XRD respectively of wheat starch and in combination with 15% cod oil, salmon oil , coconut oil and wheat gluten.

Samples	FWHM at 20 (2θ) angle	FWHM at 480 cm ⁻¹
WS	13.15 ± 0.20 ^a	10.12 ± 0.01 ^a
WS+WG	12.45 ± 0.18 ^a	11.18 ± 0.02 ^a
WS+CO	10.67 ± 0.10 ^c	5.27 ± 0.01 ^d
WS+SO	11.27 ± 0.16 ^b	8.02 ± 0.02 ^b
WS+CONT	10.38 ± 0.21 ^c	7.52 ± 0.03 ^c

WS: wheat starch; CO: cod oil; SO: salmon oil; SA: CONT: coconut oil; WG: wheat gluten. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation ($n=3$). Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

These results indicate that more V type amylose-lipid complexes were formed between WS and BCBs samples, consistent with the result of FTIR, XRD, and ¹³CNMR. The slightly lower fwhm value of WS-WG as compared to WS indicate that interaction between WS and WG was weak (Zheng et al., 2018).

12.3.5 FTIR spectra of BCBs complexes

The FTIR spectra of WS, WS-WG, and BCBs samples are an exhibit in Figure 12.2. In related to WS and WS-WG samples, three additional bands at 1643 cm^{-1} , 1744 cm^{-1} , and 2859 cm^{-1} were defined in BCBs samples FTIR spectra (Figure 12.2). The bands represent vibration of carboxylic amide derivatives ($\text{C}=\text{O}$) and $\text{C}=\text{H}$ stretching vibration of the methyl group of fatty acids respectively (Zheng et al., 2018).

Figure 12.2 FTIR spectra of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).

In BCBs samples, the peak intensity at 2859 cm^{-1} increased with short carbon chain length and decreased with increasing unsaturation of fatty acids. This indicated that formation of amylose-lipid complexes was supposed to increase the intensity of FTIR spectra of the $\text{C}=\text{H}$ stretching vibration of the methyl group of fatty acids (Wang et al., 2017). In comparison with WS and WS-WG, the more pronounced intensity peaks at 1643 cm^{-1} and 1744 cm^{-1} were recorded in the BCBs samples. The peak between $1700\text{--}1600\text{ cm}^{-1}$ and $1600\text{--}1500\text{ cm}^{-1}$ are generally characterised into amide 1 (vibration of

C=O group) and amide 2 (stretching of C=N) region of starch granules. The similar observation made by Chen et al., (2018b) who reported that peak observed at 1746 cm^{-1} due to the esterification took place between maize starch and maize oil. In BCBs sample infrared absorbance at 1643 cm^{-1} was more prominent as that of WS and WS-WG. This may be associated with vibration of the carbonyl group of amide II crystalline region of starch molecules.

12.3.6 X-ray diffraction

The X-ray diffraction analysis was usually used to attain qualitative confirmation of inclusion complex. X-ray diffraction pattern and full width height maximum (fwhm) at 20° (2θ) angle are shown in Figure 12.3 and Table 12.4.

Figure 12.3 XRD spectra of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).

All samples exhibited four diffraction peaks values of 17.5, 18, 20 and 22 at 2θ angle. WS and WS-WG showed A type crystalline peak value of 18 and 22° at a 2θ angle while BCBs sample displayed V type complex formation peaks at 2θ value of 17.5 and 20° .

Chen et al., (2018b), Farooq et al., (2018) and Wang et al., (2016) reported that the peaks observed at 19.8, 20 and 20.2 ° were considered as V type complex formation with free fatty acids while peaks between 18 and 23 ° were indicative of A type amylose-lipid complex. This indicates that the peak intensity decreased with increasing carbon chain length and saturation and increased substantially and is in general agreement with CI, DSC and FTIR result which showed that shorter carbon chain length form more complex with amylose, decreased in gelatinisation enthalpy and shifting of carbon atoms. A similar finding was made by Wang et al., (2016) who reported that WS-fatty acid mixture form V type complexes at 19.8 ° as compared to WS alone. The fwhm value of BCBs samples was exhibited significant different as compared to WS and WS-WG. The more order structure of amylose-lipid complexes was usually evaluated by fwhm value of x-ray diffraction spectra at a 2θ angle, with decreasing value indicative of more complex structure formation (Liu et al., 2018a). The BCBs sample exhibited fwhm value of 5.27-8.02 at a 2θ angle as compared to WS (10.12) and WS-WG (11.18) (Table 12.4). The result suggested that due to the presence of different saturated and unsaturated fatty acids in BCBs sample form the complex

12.3.7 *In vitro* starch digestibility

The amount of reducing sugar released over 120 min during starch digestion with different lipid sources are shown in Figure 12.4. In comparison with WS, BCBs samples exhibited a lower amount of reducing sugar released than that of WS and WS-WG. The decreased in starch digestibility in BCBs samples may be attributed to the formation of the amylose-lipid complex (Oyeyinka et al., 2017; Wang et al., 2016) and interaction of

lipid with starch inhibit the swelling of starch granules and affect the gelatinisation process (Reddy et al., 2018; Ye et al., 2018).

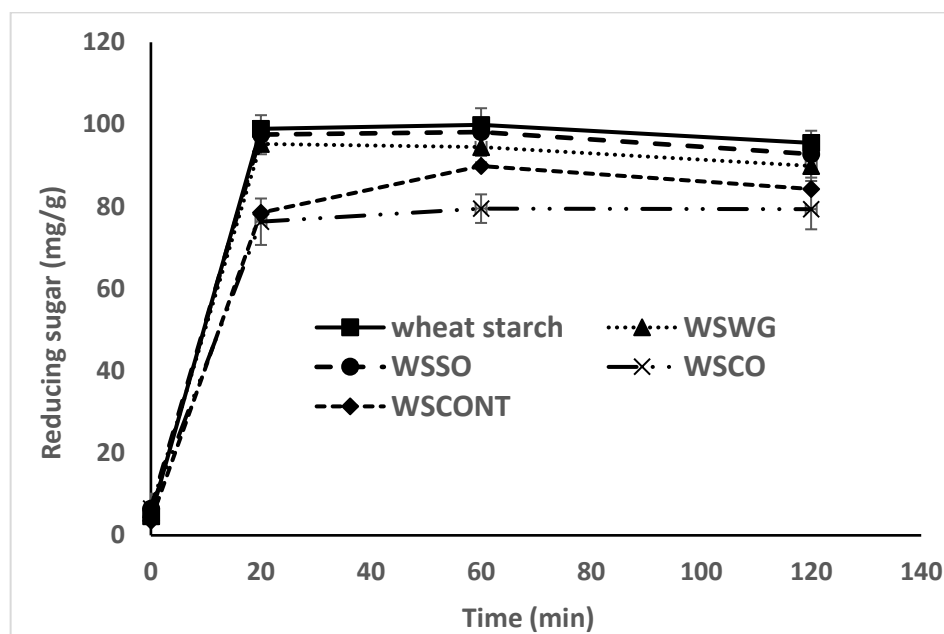


Figure 12.4 Amount of reducing sugar released during *in vitro* digestion for wheat starch and replacement of wheat starch with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CONT).

These complexes restrict the susceptibility of the enzyme to hydrolyse the starch molecules. Also, another reason may be the intermolecular hydrophobic bond present in the BCB samples are more stable and resist to starch hydrolysis than WS and WS-WG (Cheng et al., 2018). Similar finding were reported in previous reports (Ai et al., 2013; Kawai et al., 2012; Oyeyinka et al., 2017). Oyeyinka et al. (2017) reported that the starch digestion rate was decreased with complex formation between bambara starch and stearic acid. Annor et al., (2015) also studied complexing ability and starch digestibility with saturated and polyunsaturated fatty acids. Oleic acid and palmitic acid showed high complexing ability and decreased digestibility than that of linoleic acid.

12.3.8 ^{13}C nuclear magnetic response

Carbon-13 nuclear magnetic response is a tool that used to characterise the complex formation in gel with the shifting of the chemical structure of carbon atoms. The spectra and chemical shift of WS, WS-WG, and BCBs samples are shown in Table 12.5 and Figure 12.5.

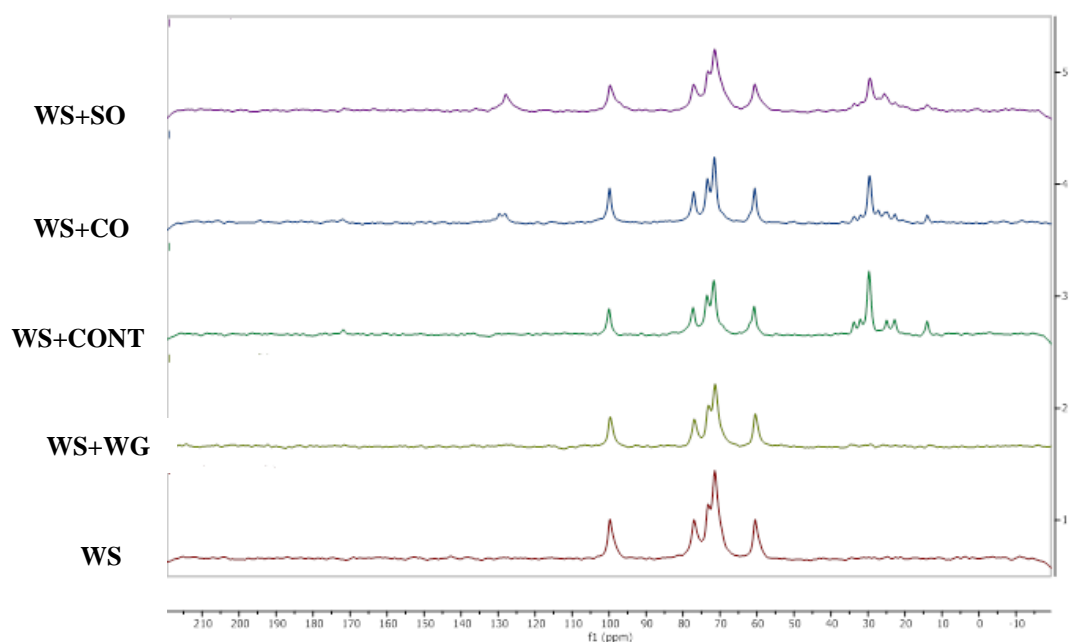


Figure 12.5 NMR spectra of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).

Table 12.5 ^{13}C - chemical shifts of wheat starch and its blend with 15% cod, salmon, coconut and wheat gluten.

Samples	Chemical shift (ppm)					
	C1	C2	C3	C4	C5	C6
WS+WG	0.00	-0.0004	-0.0009	-0.0006	-0.0009	-0.0007
WS+SO	0.001	0.001	0.001	0.002	0.001	0.001
WS+CO	0.004	0.001	0.001	0.004	0.001	0.001
WS+CONT	0.008	0.004	0.003	0.006	0.003	0.002

WS: wheat starch; CO: cod oil; SO: salmon oil; CON: coconut oil; WG: wheat gluten.

Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

WS and WS-WG samples did not show any significant difference in the resonance peaks and found in the range of 60-110 (C1-C6) ppm. In BCBs samples spectra obtained were more prominent and stable as compared to the WS and observed in the range of 60-140 (C1-C6) ppm. A starch molecule is composed of amylose (α ,1-4) linkage anhydrous glucose unit. It is reported that amylose-lipid complexes change the arrangement of a random coil amylose molecule to the helix and alter the glycosidic bonds torsion angles. The torsion angle is responsible for the distribution of electron pattern on carbon 1 and carbon 4 of the bonds which cause shifting of chemicals with downfield changes in carbon of 1 and 4 of the anhydroglucose unit in the NMR spectrum (Olivato, Müller, Carvalho, Yamashita, & Grossmann, 2014). Table 5 illustrates that greater chemical shift of carbon 1 and carbon 4 of BCBs samples as compared to WG. This result indicates that the lipid present in the starch gel formed the complexes with amylose with following order CONT>CO>SO. Subsequently, FTIR and XRD show a complex formation peak (Figure 12.2 and 12.3). Similarly, Yongfeng, Ai et al., (2013) reported that corn, tapioca, and waxy corn mixed with palmitic acid, stearic and linoleic acid form complexes with the chemical shifting of carbon 1 and carbon 4 glucose unit.

12.3.9 Morphological characteristics

The morphological structure of WS, WS-WG and BCBs sample using scanning electron microscopy (SEM) were depicted in Figure 12.6. SEM image of WS showed a smooth surface. In BCBs samples, granular structure of starch exhibited transparently and embedded with the starch surface.

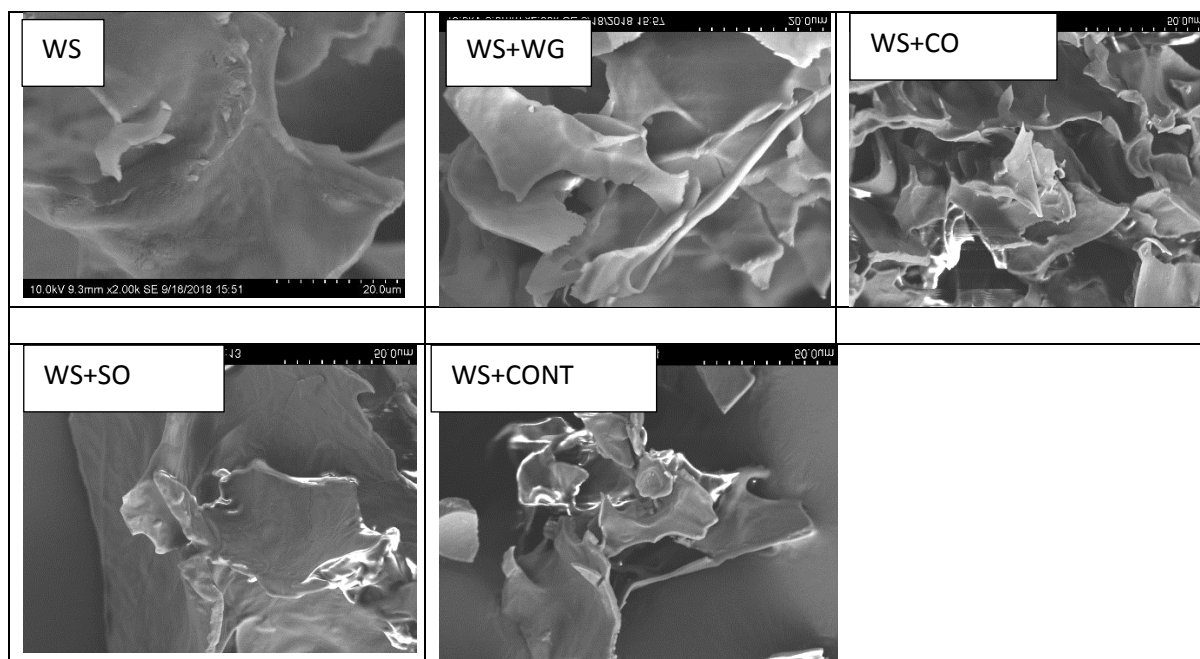


Figure 12.6 Scanning electronic microscopic (SEM) images (800 x magnification) of wheat starch (WS) and binary complex with 15 % cod oil (CO), salmon oil (SO) and coconut oil (CO) and wheat gluten (WG).

This could be attributed to complete destruction of starch granules with the addition of a short chain and polyunsaturated fatty acids during pasting. According to previous studies, gel contains saturated and unsaturated fatty acids exhibited less ordered structure of the amylose-lipid complex (Zabar, Lesmes, Katz, Shimoni, & Bianco-Peled, 2010). Also, (Okumus et al., 2018) reported that brown lentil starch mixed with stearic and olive oil showed collapsed and destruction structure of starch.

12.4 Conclusion

The effects of different lipids (salmon, cod and coconut oil) inclusion to WS on physicochemical, technological and *in vitro* digestibility properties of WS were examined. The alteration in pasting, textural and digestibility characteristics of WS were significantly influenced by the formation of amylose-lipid complexes. In comparison with WS, BCBs samples form V-type complex followed by WS-WG and showed by FTIR, XRD, Raman, and NMR. The development of complexes between amylose and lipid

addition decreased the starch swelling capacity and gelatinisation. Additionally, BCBs sample exhibited more positive impact on the reduction of starch digestibility as compared to WS and WS-WG. This could be attributed that amylose-lipid complexes inhibit the accessibility of digestive enzyme to hydrolyse the starch molecules. The finding of this study might help to consumers requiring a lower insulin response by alteration of starch physiochemical and digestibility characteristics.

Chapter 13

General discussion and conclusion

12.1 Summary

This study demonstrated that fish powder obtained from cod (*P. bachus*) and salmon (*O. tshawytscha*) could be incorporated into cereal based pasta and bread products in order to enhance the physicochemical, nutritional and sensory properties of innovative functional products. Addition of fish powder showed a positive effect on the proximate composition, physical and textural properties of pasta and bread while a higher concentration of salmon powder affected the textural quality of pasta. The results obtained support the idea that fish powder inclusion to pasta and bread products significantly impact on reducing starch digestibility, protein digestibility, increasing amino acid and fatty acid profile, TPC and antioxidant activity and sensory quality. Incorporation of fish powder to pasta and bread products not only increase the physicochemical and nutritional properties but also accepted by consumer panel though sensory evaluation. Cod protein acted as barrier between the starch and α -amylase resulting in a decrease of reducing sugar release of pasta and bread while salmon powder acted as a ternary complexing agent in which the amylose-lipid-protein formed complex that inhibiting starch from gelatinisation and altering the structure of samples.

12.2 Discussion

The addition of fish powder increased the proximate composition (protein, lipid, energy) of the pasta and bread products. Chapters 4 and 6 present the proximate analysis of pasta and bread prepared with cod and salmon powder. Pasta and bread

made with different levels (5, 10 15 and 20%) of fish powder showed significantly increased protein, lipid and energy contents while moisture and carbohydrate contents were reduced. A similar trend was observed by Monteiro et al. (2018) and Lorusso et al., (2017). This increase in protein, lipid and energy value in pasta and bread enriched with fish powder could be due to the inclusion of nutrients such as protein, polyunsaturated fatty acids and essential amino acids present in the fish powder (Khalili Tilami & Sampels, 2018) but absent from the durum wheat semolina and wheat (Zhang et al., 2016). The decrease in the moisture content could to be attributed to a change in the protein-starch interaction when compared to control pasta; the interaction between the different and increased proteins and the starch might have resulted into the entrapment of water molecules through electrostatic forces, and subsequently the more homogenous network developed with less free water (Zhang et al., 2016). It was found that inclusion of fish powder affected the starch-protein network and competing nature of fish protein and lipid to water during cooking had an impact on cooking properties of pasta. The cooking quality of pasta is an important feature and is assessed using optimal cooking time, cooking loss (solid material leaching during cooking), water absorption index, and swelling index which represents the uptake of water content during cooking. Chapters 4 and 6 present information on the cooking quality attributes of pasta made with fish powder (cod and salmon). In this study, there was an increase in the cooking loss recorded on adding fish powder. The higher cooking loss in pasta enriched with fish powder might be attributed to a weakening and disruption of the protein gluten network. Similar finding were observed by Ramya et al., (2015) and Tazart et al., (2016).

In this study, the pasta made with different levels of (5, 10, 15 and 20%) fish powder exhibited a reduction in swelling index compared to the control (Chapters 4 and 6). The reduced swelling index could be due to the formation of a strong protein network and starch-lipid complex in the pasta resulting in the limited supply of water for starch granule for swelling and gelatinisation. This is in agreement with results obtained by (Liu et al., 2016). Wang et al. (2016) reported that formation of starch-lipid complexes prevents leaching of amylose from starch granules during gelatinisation. The nature of ingredients used and degree of starch gelatinisation could be responsible for decrease in swelling index as reported by other researchers (Brennan et al., 2012b). The water absorption index (WAI) of pasta enriched with fish powder reduced compared to control pasta (Chapters 4 and 6). Brennan et al., (2004) reported that the water absorption index of pasta increased due to the increased degree of starch gelatinisation and disruption of the protein-starch matrix within the product. Substitution of semolina flour with fish powder reduces starch swelling and pasta water absorption by competing with the starch for water and formation of weak protein matrix through starch-lipid complex during pasta formation. These results are in agreement with Bagdi et al. (2016) and Lorusso et al. (2017) who reported that pasta enriched with aleurone rich flour and quinoa flour had significantly decreased WAI compared to wheat only pasta. Similarly, Ramya et al. (2015) and Vijaykrishnaraj et al. (2014) reported that the addition of shrimp meat and green mussel powder to pasta decreased WAI as the level of shrimp meat and green mussel powder increased. In our study, it has been observed that incorporation of cod fish powder had increased firmness and extensibility of pasta product compared to the control pasta (Chapter 4). The textural properties of pasta are mainly controlled by a gluten network, which is a structural network of starches, protein additions, and

other ingredients (Chang & Wu, 2008). It seems possible that fish protein interacts with the insoluble network of pasta, forming a matrix structure, and leading to an increased firmness and extensibility as observed in this study. Textural parameters increased might be attributed to the higher amount of polypeptide chain associated with higher protein content, which increases the ability of proteins to form an insoluble network and entrap swollen and gelatinised starch granules that prevents pasta from disruption (Chillo et al., 2010). The textural properties of pasta in the this study correspond to results from studies carried out on the addition of shrimp meat (Kadam & Prabhasankar, 2012). The firmness and extensibility of pasta made with salmon powder was low compared to control pasta (Chapter 5). It might be possible that high cooking loss in enriched pasta was mainly caused by their lipid content which weakened the gluten network of pasta and made the pasta soft. This result is in agreement with other work carried out by Vijaykrishnaraj et al., (2014) and Giuberti et al., (2015). Weakening of the pasta structure in this study could be due to the interference of starch granules by lipids and inhibition of the solubilisation of starch and the subsequent disturbance of the starch–protein matrix (Lu et al., 2009). In bread, the addition of fish powder increased hardness, gumminess and chewiness attributes while springiness, cohesiveness, and resilience were found to be decreased (Chapters 9 and 10). This might be due to the thickening of gas cell walls within the bread crumb due to the high water absorption characteristics of fish protein powder. Additionally, the increased protein and decreased lipid content in bread enriched with cod powder might interfere with gas retention and create more unstable gas cells resulting in a compact structure giving it a chewy crumb making it harder. Phongthai et al. (2016) and Fagundes et al. (2018) reported that a 4 % inclusion of egg albumin and 12 % cobia fish protein resulted in a

significant increase in bread firmness. Lu et al. (2018a), who reported that addition of mushroom powder led to a lower bread springiness and cohesiveness value.

Starch digestibility and predicted glycaemic response of food product mainly depend on product type (food matrix), processing conditions (mixing, cooking, extrusion) and ingredients used in the product (protein and lipid) (Bello-Perez, Flores-Silva, Agama-Acevedo, & Tovar, 2018). In this study, (Chapters 5, 7, 9, 10) the addition of CFP and SFP have lowered the reducing sugar release of pasta and bread. Starch digestibility rate was found to be higher in control sample than in samples enriched with CFP and SFP. The lower values were observed in pasta and bread with inclusion of 5-20 % CFP and SFP. This could be due to the addition of fish powder creating a protein network around the starch molecules and reduce the starch granules' surface accessibility of α -amylase to starch and, hence, affect the enzyme's ability to hydrolyse the starch into reducing sugar. Recently published results in other products (Hager et al., 2013; Liu et al., 2016; Ramya et al., 2015) have similar findings. The observation may be related to the fact that lipids form complexes with amylose resulting in the formation of single helical structure with a conformational hindrance that restricts enzymes to hydrolyse the starch granule and protein blocked enzyme adsorption sites on the surface of starch granule (Chen et al., 2017a). Lipids present in pasta and bread products, interact with amylose and prevent the starch granules from swelling and thus reduce the starch gelatinisation process which decreases the starch digestibility. Similarly, the predicted glycaemic response (Chapters 5, 7, 9, 10) was reduced after the incorporation of CFP and SFP. The addition of 5-20 % CFP and SFP to pasta and bread products decreased the value of the AUC. The alteration in AUC value could be linked with protein and lipid content of fish powder. The composition of CFP and SFP could be reason for lowered

glycaemic response. In this it can be suggested that the incorporation of protein-rich ingredients into pasta could modify the integrity of the protein network and entrap α -amylase activity (Liu et al., 2016). Similar results were reported when investigating the *in vitro* starch digestibility and glycaemic index of millets with different fatty acids palmitic, oleic and linoleic acids (Annor et al., 2015). Protein quality is arguably the most important attribute to determine the nutritional characteristics of a food matrix and is evaluated by *in vitro* protein digestibility, PDCAAS and DIAAS. In this study, (Chapter 5, 7, 9 & 10) protein digestibility of pasta enriched with CFP and SFP decreased compared to the control samples. This could be due to the interaction of phenolic compounds with protein which might change the digestibility. It has been reported that oxidized phenolic compounds might react with proteins and form insoluble complexes which inhibit the activity of proteolytic enzymes and interfering with utilisation of proteins (Labuckas et al., 2008). Similar observations were reported by Ramya et al., (2015) and Rodríguez De Marco et al., (2014). Also, due to pasta cooking process, the anti-nutritional factor present in semolina decreases and alters the protein digestibility value (de la Peña & Manthey, 2014). The amino acid profile of the digested sample was correlated with IVPD and revealed that the decrease in amino acid availability could be due to interactions of protein and lipid - phenolic compounds in pasta resulting in the oxidation of proteins and amino acids rendering them unavailable to proteolytic enzyme hydrolysis and subsequent intestinal absorption. A similar observation was made by Liu et al., (2016). Increasing the percentage of SFP in the pasta formulation significantly enhanced EPA and DHA fatty acid content (Chapter 8). In turn, the pasta prepared with SFP presented an important proportion of this LC-PUFAs in relation to other fatty acids and it was possible to obtain an important reduction of the ratio ω -6: ω -3 (5:1) in comparison with

the control pasta. The total IAA contents (mg IAA/g protein) of all the pasta samples exceeded the recommended daily allowance (277 mg IAA/g protein) and contributed on average 41 % to TAA contents (Chapter 8). The control pasta contained a relatively low proportion of IAA to TAA (27.5 %) among the pasta enriched with 5-20 % SFP. Furthermore, IVPD and amino acid profile of bread enriched with CFP and SFP was found to be increased. This may be because the fermentation process accelerated the proteolysis. Similar findings were reported by Arte et al., (2015). This is in agreement with work carried out by Villarino, Jayasena, Coorey, Chakrabarti-Bell, Foley, et al. (2015a) who reported that legume lupin (*Lupinus angustifolius*) flour addition in bread increases the protein digestibility. In this study, the essential amino acids (lysine, leucine, isoleucine, methionine, tyrosine, threonine, and valine), fatty acid profile (DHA and EPA), PDCAAS and nutritional index (EAAI, BV and NI indices) of the bread were increased as compared to the control bread (Chapters 9 and 10). It has been reported that wheat flour is deficient in one or more essential amino acid like lysine and SFP addition into bread might be fulfil this requirement and enhance the nutritional quality of bread. Similar findings were reported by Lorusso et al., (2017). Cereals are rich in linoleic acid ($n-6$) which make them imbalanced to $\omega-6$: $\omega-3$ ratio (Fradique et al., 2013). Increasing the percentage of SFP significantly enhanced EPA and DHA fatty acid content and maintained $\omega-6$: $\omega-3$ ratio as recommended by European Food Safety Authority (EFSA), (2010). Consumption of food with phenolic rich ingredients is highly recommended due to their health promoting effects in the prevention of cancers, diabetes and cardiovascular diseases. Bio-accessibility of the phenolic content and antioxidant capacity of food product is important from a nutritional point of view. For determination of bio-accessibility, bioactive compounds released from the food matrix

during digestion and available to absorption need to be considered (Ma et al., 2014). In this study, pasta enriched with CFP and SFP showed significantly increased bio-accessibility of phenolic content and antioxidant activity (ORAC) after gastric and pancreatic digestion (Chapters 5, 7, 9, & 10). The highest values were obtained for pasta fortified with a 20 % supplement. The low value observed in control pasta could be due to the leaching of phenolic compounds into the cooking medium. However, in the case of enriched pasta, retention of phenolic content was significantly higher. This clearly indicates that incorporation of fish powder results in the retention of phenolic compounds in the pasta upon cooking. Similar findings were made by Fares et al., (2010), Khan et al., (2013) and Prabhasankar et al., (2009). Additionally, the presence of oxygen, water, and heat treatment during cooking and pasta making may induce the oxidation of sensitive phenolic antioxidants (Khan et al., 2013). Bread fortified with SFP showed a decrease in the phenolic content and antioxidant capacity. This might be due to the thermal deactivation of phenolic compound during baking process and formation of indigestible complexes with SFP protein and lipid, which agrees with previous studies such as the enrichment of bread with quinoa leaves (Swieca et al., 2014). Also, masking of antioxidant activity of bread could be attributed to phenolics compounds present in the SFP that might have formed protein-phenolics or phenolic-lipid complexes via hydrogen and /or hydrophilic interactions (Swieca et al., 2013).

Sensory evaluation is consider to be the best method for evaluation of the acceptability of pasta and bread products (Bustos , Perez, & León, 2011). In this study, six parameters were evaluated including appearance, colour, aroma, taste, texture and overall quality and the results indicate that all the attributes were above the minimum limit for acceptability (Chapter 7). Appearance and colour did not show a significant difference

between control samples and enriched pasta samples with 5 % and 15 % CFP and SFP respectively. For acceptability of taste, aroma, texture, and overall quality, the control pasta and pasta at 5 % cod and 5 % salmon powder incorporation scored in the range of “like slightly” while pasta fortified with 15 % cod and 15 % salmon powder scored in the range of “neither like nor dislike”. Similar findings were observed by Kadam & Prabhasankar, (2012) and Ramya et al., (2015).

The matrix of stable food is composed of starch, lipid, and protein in different concentrations with other minor constituents. During food processing, the complex interaction of these micronutrients take place and affect the physicochemical (food texture, viscosity, colour) and nutritional (starch digestibility/ glycaemic response), structural and organoleptic properties of finished food products (Parada & Santos, 2016; Zheng et al., 2018). Consumption of highly digestible starchy food leads to the outbreak of many chronic diseases such as diabetes and obesity and therefore, food containing slowly digestible starch and resistant starch have been recommended to prevent such types of disease. The starch digestion depends on its linear chain amylose, branched chain amylopectin content and complex interaction of starch with lipids and proteins in the food matrix (Annor et al., 2015). In this study, the interaction of different lipid sources salmon oil (SO), cod oil (CO), coconut oil (CONT) and protein (gluten) with wheat starch (WS) and its effect on functional and *in vitro* digestibility of starch were analysed (Chapter 11 and 12). Chapter 11 and 12 focused on the ternary and binary complex formation between starch-protein-lipid and starch-lipid present in the starch gel structure. The result indicated that fatty acids present in the different oils form the complex with amylose and gluten (ternary) and amylose-lipid (binary) and exhibited a decreased peak viscosity, an increased pasting temperature and decreased enthalpy

changes. SEM images showed the more embedded and destruction structure of starch granules in ternary and binary samples as compared to WS due to the complex formation. The colour and textural indices of ternary and binary samples were found to be significantly decreased than that of WS alone. The findings of Raman and Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and ^{13}C NMR techniques showed that the structural changes occurred in ternary and binary samples with more ordered structure of complex formation with starch-lipid- protein and starch-lipid. Results displayed that a significant decreased in starch digestibility in ternary and binary samples as compared to WS in order of CO>CONT>SO>WG>WS (chapter 11 and 12). This results demonstrated that inclusion of short chain saturated fatty acid and unsaturated fatty acids to WS inhibits the activity of starch swelling and gelatinisation (Ye et al., 2018). Also, starch-protein-lipid/starch-lipid interaction in starch gel form the V type complexes that inhibit the ability of digestive enzymes to hydrolyse the starch molecules (Chapter 11 and 12).

12.3 Recommendation for future work

This study demonstrated that fish powder exhibits a positive benefit in terms of improving the physiochemical and nutritional properties of cereal products. Inclusion of fish powder to pasta and bread products had increased firmness and extensibility. A significant effect of fish powder was observed in the reduction of glucose in the *in vitro* analysis, IVPD was influenced by addition of fish powder, increased the bio-accessibility of phenolic and antioxidant capacity and consumer acceptability of pasta products proved that there is enormous potential of using fish powder as a value added ingredients to alter the nutritional characteristics of the cereal products. For the

confirmation of *in vitro* analysis result in this study it is necessary to conduct *in vivo* analysis. During production of fish powder from fresh raw material, the waste material including fish skin, fins, head and bones treated as waste material which accounts up to 40 % of fish weight. Thus, this material could be processed and incorporated into snack products in order to reduce waste disposal cost substantially and develop a low cost and nutritionally rich product.

Appendix

A.1 Approval letter from Human Ethics Committee

Research Management Office

T 64 3 423 0817
PO Box 85084, Lincoln University
Lincoln 7647, Christchurch
New Zealand
www.lincoln.ac.nz

8 December 2017

Application No: 2017-49

Title: Sensory evaluation of pasta

Applicant: A.S. Desai, Ph.D. Student

The Lincoln University Human Ethics Committee has reviewed the above noted application. Thank you for your response to the questions which were forwarded to you on the Committee's behalf. I am satisfied on the Committee's behalf that the issues of concern have been satisfactorily addressed. I am pleased to give final approval to your project. I strongly recommend that you seek advice on editing the three documents to go to potential participants as they contain numerous written expression errors. Please note that this approval is valid for three years from today's date at which time you will need to reapply for renewal. Once your field work has finished can you please advise the Human Ethics Secretary, Alison Hind, and confirm that you have complied with the terms of the ethical approval. May I, on behalf of the Committee, wish you success in your research.

Yours sincerely



Caitriona Cameron
Acting Chair, Human Ethics Committee

A.2 Questionnaires' for sensory analysis

Enter your **first sample number** here _____

➤ Appearance

Please tick **ONE** box that best describes your liking for appearance of the sample.

Dislike Extremely	Dislike very much	Dislike moderat ely	Dislike slightly	Neither like or dislike	Like slightly	Like moder ately	Like very much	Like Extre mely

➤ Colour

Please tick **ONE** box that best describes your liking for colour of the sample.

Dislike Extremely	Dislike very much	Dislike moderat ely	Dislike slightly	Neither like or dislike	Like slightly	Like mode rately	Like very much	Like Extre mely

➤ Taste

Please tick **ONE** box that best describes your liking for taste

Dislike Extremely	Dislike very much	Dislike moderat ely	Dislike slightly	Neither like or dislike	Like slightly	Like modera tely	Like very much	Like Extre mely

➤ Aroma

Please tick **ONE** box that best describes your liking for aroma of the sample.

Dislike Extremely	Dislike very much	Dislike moderat ely	Dislike slightly	Neither like or dislike	Like slightly	Like modera tely	Like very much	Like Extre mely

➤ Texture

Please tick **ONE** box that best describes your liking for texture

Dislike Extremely	Dislike very much	Dislike moderat ely	Dislike slightly	Neither like or dislike	Like slightly	Like modera tely	Like very much	Like Extre mely

➤ Overall acceptability

Please tick **ONE** box that best describes your overall liking

Dislike Extremely	Dislike very much	Dislike moderat ely	Dislike slightly	Neither like or dislike	Like slightly	Like modera tely	Like very much	Like Extre mely

Thank you very much for your participation!

A.3 QR analysis data

Recorded Date	Distribution Channel	Age	gender	Sample code no.	Apperance	Colour	Taste	Aroma	Texture	Overall quality
12/03/2018	qr	25 - 34	Male	128	Like Extremely	Like moderately	Neither like or dislike	Like slightly	Like slightly	Like slightly
12/03/2018	qr	25 - 34	Male	526	Like very much	Like very much	Like slightly	Like slightly	Like slightly	Like slightly
12/03/2018	qr	18 - 24	Male	715	Like slightly	Neither like or dislike	Dislike moderately	Dislike very much	Dislike slightly	Dislike moderately
12/03/2018	qr	25 - 34	Male	216	Like very much	Like very much	Neither like or dislike	Dislike slightly	Neither like or dislike	Like slightly
12/03/2018	qr	35 - 44	Male	128	Like slightly	Like moderately	Like slightly	Dislike slightly	Like moderately	Like slightly
12/03/2018	qr	25 - 34	Female	443	Neither like or dislike	Dislike slightly	Dislike slightly	Dislike slightly	Dislike slightly	Dislike slightly
12/03/2018	qr	55 - 64	Female	715	Like moderately	Like moderately	Dislike very much	Dislike moderately	Dislike slightly	Dislike moderately
12/03/2018	qr	35 - 44	Male	443	Like moderately	Dislike slightly	Like moderately	Like moderately	Like very much	Like moderately
12/03/2018	qr	25 - 34	Female	526	Like moderately	Like slightly	Like moderately	Like moderately	Like very much	Like very much
12/03/2018	qr	25 - 34	Female	715	Like Extremely	Like Extremely	Like slightly	Like moderately	Like very much	Like very much
12/03/2018	qr	25 - 34	Female	526	Neither like or dislike	Like slightly	Dislike moderately	Dislike moderately	Dislike slightly	Dislike slightly

12/03/2018	qr	35 - 44	Female	128	Neither like or dislike	Like slightly	Dislike slightly	Neither like or dislike	Dislike moderately	Dislike moderately
12/03/2018	qr	35 - 44	Male	443	Like Extremely	Like very much	Like very much	Like very much	Like very much	Like very much
12/03/2018	qr	25 - 34	Male	216	Like slightly	Like moderately	Like slightly	Like very much	Like slightly	Like moderately
12/03/2018	qr	25 - 34	Female	128	Like moderately	Like very much	Dislike moderately	Dislike very much	Neither like or dislike	Like slightly
12/03/2018	qr	35 - 44	Male	216	Dislike Extremely	Dislike Extremely	Dislike moderately	Dislike moderately	Dislike moderately	Dislike moderately
12/03/2018	qr	25 - 34	Female	526	Neither like or dislike	Neither like or dislike	Dislike slightly	Dislike slightly	Neither like or dislike	Dislike slightly
12/03/2018	qr	35 - 44	Male	128	Like slightly	Like slightly	Dislike moderately	Dislike slightly	Like moderately	Dislike slightly
12/03/2018	qr	25 - 34	Male	216	Like moderately	Like very much	Like slightly	Dislike very much	Like moderately	Dislike slightly
12/03/2018	qr	45 - 54	Male	715	Like very much	Like moderately	Like very much	Like slightly	Like Extremely	Like very much
12/03/2018	qr	25 - 34	Male	443	Like very much	Like moderately	Like very much	Like very much	Like very much	Like very much
12/03/2018	qr	35 - 44	Female	128	Dislike very much	Neither like or dislike	Dislike very much	Like slightly	Dislike moderately	Dislike very much
12/03/2018	qr	35 - 44	Male	526	Like slightly	Like slightly	Dislike slightly	Dislike slightly	Like slightly	Neither like or dislike
12/03/2018	qr	25 - 34	Male	128	Like slightly	Like slightly	Dislike very much	Dislike very much	Dislike slightly	Dislike very much
12/03/2018	qr	25 - 34	Male	443	Like moderately	Like moderately	Dislike moderately	Neither like or dislike	Neither like or dislike	Dislike slightly

14/03/2018	qr	18 - 24	Female	128	Like moderately	Like moderately	Like moderately	Like moderately	Like moderately	Like very much
14/03/2018	qr	25 - 34	Female	715	Like Extremely	Like Extremely	Like moderately	Like very much	Like very much	Like Extremely
14/03/2018	qr	18 - 24	Female	216	Like moderately	Dislike moderately	Dislike slightly	Dislike moderately	Like moderately	Dislike slightly
14/03/2018	qr	35 - 44	Male	443	Like moderately	Like slightly	Neither like or dislike	Dislike slightly	Neither like or dislike	Like slightly
14/03/2018	qr	55 - 64	Female	443	Neither like or dislike	Like slightly	Neither like or dislike	Like slightly	Like slightly	Like slightly
14/03/2018	qr	25 - 34	Male	443	Like moderately	Like moderately	Like very much	Neither like or dislike	Like moderately	Like moderately
14/03/2018	qr	25 - 34	Male	715	Like very much	Like moderately	Like very much	Like slightly	Like slightly	Like moderately
14/03/2018	qr	45 - 54	Male	128	Like moderately	Like slightly	Dislike moderately	Dislike moderately	Like moderately	Dislike moderately
14/03/2018	qr	55 - 64	Male	715	Neither like or dislike	Neither like or dislike	Dislike slightly	Dislike slightly	Dislike slightly	Dislike moderately
14/03/2018	qr	25 - 34	Female	443	Like moderately	Dislike moderately	Dislike very much	Dislike very much	Dislike slightly	Dislike very much
14/03/2018	qr	65 - 74	Male	128	Dislike slightly	Like very much	Like moderately	Like moderately	Like very much	Like moderately
14/03/2018	qr	65 - 74		216	Like moderately	Like moderately	Like moderately	Like moderately	Like slightly	Like moderately
14/03/2018	qr	25 - 34	Male	715	Like very much	Like moderately	Like moderately	Like moderately	Like moderately	Like moderately
14/03/2018	qr	35 - 44	Male	128	Like moderately	Like moderately	Neither like or dislike	Neither like or dislike	Like moderately	Like slightly

14/03/2018	qr	25 - 34	Male	128	Like moderately	Like very much	Like moderately	Like very much	Like slightly	Like moderately
------------	----	---------	------	-----	-----------------	----------------	-----------------	----------------	---------------	-----------------

References

- AACC, 2010. Approved Methods of the American Association of Cereal Chemists (10th ed.) method 10-05.01. St. Paul, MN, U.S.A. <http://dx.doi.org/10.1094/AACCIIntMethod-10-05.01>
- Aadland, E. K., Graff, I. E., Lavigne, C., Eng, O., Paquette, M., Holthe, A., ... Liaset, B. (2016). Lean seafood intake reduces postprandial c-peptide and lactate concentrations in healthy adults in a randomized controlled trial with a crossover design. *Journal of Nutrition*, 146(5), 1027–1034. <https://doi.org/10.3945/jn.115.229278>
- Agostoni, C., Bresson, J. L., & Chardigny, J. M. (2010). Outcome of the public consultation on the draft opinion of the scientific panel on dietetic products, nutrition, and allergies (NDA) on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated. *European Food Safety Authority Journal*, 8(5), 1–232. <https://doi.org/10.2903/j.efsa.2010.1507>.
- Ahn, C. B., Kim, J. G., & Je, J. Y. (2014). Purification and antioxidant properties of octapeptide from salmon byproduct protein hydrolysate by gastrointestinal digestion. *Food Chemistry*, 147, 78–83. <https://doi.org/10.1016/j.foodchem.2013.09.136>
- Ai, Y., Hasjim, J., & Jane, J. L. (2013). Effects of lipids on enzymatic hydrolysis and physical properties of starch. *Carbohydrate Polymers*, 92(1), 120–127. <https://doi.org/10.1016/j.carbpol.2012.08.092>
- Akeson, W. R., & Stahmann, M. A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *The Journal of Nutrition*, 83(7), 257–261.
- Akinwale, T. E., Shittu, T. A., Adebawale, A. A., Adewuyi, S., & Abass, A. B. (2017). Effect of soy protein isolate on the functional, pasting, and sensory acceptability of cassava starch-based custard. *Food Science and Nutrition*, 5(6), 1163–1169. <https://doi.org/10.1002/fsn3.507>
- Almanza-Benitez, S., Osorio-Díaz, P., Méndez-Montealvo, G., Islas-Hernández, J. J., & Bello-Perez, L. (2015). Addition of acid-treated unripe plantain flour modified the

- starch digestibility, indigestible carbohydrate content and antioxidant capacity of semolina spaghetti. *LWT - Food Science and Technology*, 62(2), 1127–1133. <https://doi.org/10.1016/j.lwt.2015.02.031>
- Anbudhasan, P., & Surendraraj, A. (2014). Development of functional pasta enriched with Omega 3 fatty acids. *Fishery Technology*, 10(1), 1–6.
- Annor, G. A., Marcone, M., Bertoft, E., & Seetharaman, K. (2013). *In vitro* starch digestibility and expected glycemic index of kodo millet (*Paspalum scrobiculatum*) as affected by starch-protein-lipid interactions. *Cereal Chemistry*, 90(3), 211–217. <https://doi.org/10.1094/CCHEM-06-12-0074-R>
- Annor, G. A., Marcone, M., Corredig, M., Bertoft, E., & Seetharaman, K. (2015). Effects of the amount and type of fatty acids present in millets on their *in vitro* starch digestibility and expected glycemic index (eGI). *Journal of Cereal Science*, 64, 76–81. <https://doi.org/10.1016/j.jcs.2015.05.004>
- Aravind, N., Sissons, M., Egan, N., Fellows, C. M., Blazek, J., & Gilbert, E. P. (2012a). Effect of β -glucan on technological, sensory, and structural properties of durum wheat pasta. *Cereal Chemistry*, 89(2), 84–93. <https://doi.org/10.1094/CCHEM-08-11-0097>
- Aravind, N., Sissons, M. J., Fellows, C. M., Blazek, J., & Gilbert, E. P. (2012b). Effect of inulin soluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. *Food Chemistry*, 132(2), 993–1002. <https://doi.org/10.1016/j.foodchem.2011.11.085>
- Arte, E., Rizzello, C. G., Verni, M., Nordlund, E., Katina, K., & Coda, R. (2015). Impact of enzymatic and microbial bioprocessing on protein modification and nutritional properties of wheat bran. *Journal of Agricultural and Food Chemistry*, 63(39), 8685–8693. <https://doi.org/10.1021/acs.jafc.5b03495>
- Aryee, A. N. A., & Boye, J. I. (2016). Improving the digestibility of lentil flours and protein isolate and characterization of their enzymatically prepared hydrolysates. *International Journal of Food Properties*, 19(12), 2649–2665. <https://doi.org/10.1080/10942912.2015.1123269>
- Augustin, L. S. A., Kendall, C. W. C., Jenkins, D. J. A., Willett, W. C., Astrup, A., Barclay, A. W., ... Poli, A. (2015). Glycemic index, glycemic load and glycemic response: An international scientific consensus summit from the international carbohydrate

- quality consortium (ICQC). *Nutrition, Metabolism and Cardiovascular Diseases*, 25(9), 795–815. <https://doi.org/10.1016/j.numecd.2015.05.005>
- Bagdi, A., Toth, B., Lorincz, R., Szendi, S., Gere, A., Kokai, Z., ... Tomoskozi, S. (2016). Effect of aleurone-rich flour on composition, baking, textural, and sensory properties of bread. *LWT - Food Science and Technology*, 65, 762–769. <https://doi.org/10.1016/j.lwt.2015.08.073>
- Baghurst, K. (2005). Nutrient reference values for Australia and New Zealand including recommended dietary intakes. *Nutrition*, 2, 1-254
- Baiano, A., Lamacchia, C., Fares, C., Terracone, C., & La Notte, E. (2011). Cooking behaviour and acceptability of composite pasta made of semolina and toasted or partially defatted soy flour. *LWT - Food Science and Technology*, 44(4), 1226–1232. <https://doi.org/10.1016/j.lwt.2010.11.029>
- Bannenberg, G., Mallon, C., Edwards, H., Yeadon, D., Yan, K., Johnson, H., & Ismail, A. (2017). Omega-3 long-chain polyunsaturated fatty acid content and oxidation state of fish oil supplements in New Zealand. *Scientific Reports*, 7(1), 1488–1501. <https://doi.org/10.1038/s41598-017-01470-4>
- Baum, S. J., Kris-Etherton, P. M., Willett, W. C., Lichtenstein, A. H., Rudel, L. L., Maki, K. C., ... Block, R. C. (2012). Fatty acids in cardiovascular health and disease: A comprehensive update. *Journal of Clinical Lipidology*, 6(3), 216–234. <https://doi.org/10.1016/j.jacl.2012.04.077>
- Beara, I. N., Lesjak, M. M., Orcic, D. Z., Simin, N. D., Cetojevic-Simin, D. D., Bozin, B. N., & Mimica-Dukic, N. M. (2012). Comparative analysis of phenolic profile, antioxidant, anti-inflammatory and cytotoxic activity of two closely-related plantain species: *Plantago altissima* L. and *Plantago lanceolata* L. *LWT - Food Science and Technology*, 47(1), 64–70. <https://doi.org/10.1016/j.lwt.2012.01.001>
- Bello-Perez, L. A., Flores-Silva, P. C., Agama-Acevedo, E., & Tovar, J. (2018). Starch digestibility: past, present, and future. *Journal of the Science of Food and Agriculture*, 3(2), 1–8. <https://doi.org/10.1002/jsfa.8955>
- Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing (antioxidant) power as a measure of antioxidant capacity: the FRAP assay. *Methods in Enzymology*, 299, 15–36.
- Bhattacharya, S., Das, H., & Bose, A. N. (1988). Effect of extrusion process variables on *in-vitro* protein digestibility of fish-wheat flour blends. *Food Chemistry*, 28(3), 225–

231. [https://doi.org/10.1016/0308-8146\(88\)90054-4](https://doi.org/10.1016/0308-8146(88)90054-4)
- Bhattarai, R. R., Dhital, S., & Gidley, M. J. (2016). Interactions among macronutrients in wheat flour determine their enzymic susceptibility. *Food Hydrocolloids*, 61, 415–425. <https://doi.org/10.1016/j.foodhyd.2016.05.026>
- Bhupathiraju, S. N., Tobias, D. K., Malik, V. S., Pan, A., Hruby, A., Manson, J. E., ... Hu, F. B. (2014). Glycemic index , glycemic load , and risk of type 2 diabetes : results from 3 large US cohorts and an updated meta-analysis 1- 3. *The American Journal of Clinical Nutrition*, 100(7), 218–232. <https://doi.org/10.3945/ajcn.113.079533>
- Biernacka, B., Dziki, D., Gawlik-Dziki, U., Rozyło, R., & Siastała, M. (2017). Physical, sensorial, and antioxidant properties of common wheat pasta enriched with carob fiber. *LWT - Food Science and Technology*, 77, 186–192. <https://doi.org/10.1016/j.lwt.2016.11.042>
- Borczak, B., Sikora, M., Sikora, E., Dobosz, A., & Kapusta-Duch, J. (2017). Glycaemic index of wheat bread. *Starch - Starke*, 70, 1–11. <https://doi.org/10.1002/star.201700022>
- Borzoei, S., Neovius, M., Barkeling, B., Teixeira-Pinto, A., & Rossner, S. (2006). A comparison of effects of fish and beef protein on satiety in normal weight men. *European Journal of Clinical Nutrition*, 60(7), 897–902. <https://doi.org/10.1038/sj.ejcn.1602397>
- Bouasla, A., Wojtowicz, A., & Zidoune, M. N. (2017). Gluten-free precooked rice pasta enriched with legumes flours: Physical properties, texture, sensory attributes and microstructure. *LWT - Food Science and Technology*, 75, 569–577. <https://doi.org/10.1016/j.lwt.2016.10.005>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Brennan, C., Brennan, M., Derbyshire, E., & Tiwari, B. K. (2011). Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science and Technology*, 22(10), 570–575. <https://doi.org/10.1016/j.tifs.2011.05.007>
- Brennan, C. S., Blake, D. E., Ellis, P. R., & Schofield, J. D. (1996). Effects of guar galactomannan on wheat bread microstructure and on the *in vitro* and *in vivo* digestibility of starch in bread. *Journal of Cereal Science*, 24(2), 151–160. <https://doi.org/10.1006/jcrs.1996.0048>

- Brennan, C. S., & Cleary, L. J. (2005). The potential use of cereal (1→3,1→4)-β-d-glucans as functional food ingredients. *Journal of Cereal Science*, 42(1), 1–13. <https://doi.org/10.1016/j.jcs.2005.01.002>
- Brennan, C. S., Kuri, V., & Tudorica, C. M. (2004). Inulin-enriched pasta: Effects on textural properties and starch degradation. *Food Chemistry*, 86(2), 189–193. <https://doi.org/10.1016/j.foodchem.2003.08.034>
- Brennan, C. S., & Tudorica, C. M. (2007). Fresh pasta quality as affected by enrichment of nonstarch polysaccharides. *Journal of Food Science*, 72(9), 658–665. <https://doi.org/10.1111/j.1750-3841.2007.00541.x>
- Brennan, Derbyshire, E., Tiwari, B. K., & Brennan, C. S. (2013a). Integration of beta-glucan fibre rich fractions from barley and mushrooms to form healthy extruded snacks. *Plant Foods For Human Nutrition*, 68(1), 78–82. <https://doi.org/10.1007/s11130-012-0330-0>
- Brennan, Derbyshire, E., Tiwari, B. K., & Brennan, C. S. (2013b). Ready-to-eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. *International Journal of Food Science & Technology*, 48(5), 893–902. <https://doi.org/10.1111/ijfs.12055>
- Brennan, M. A., Derbyshire, E., Tiwari, B. K., & Brennan, C. S. (2012a). Enrichment of extruded snack products with coproducts from chestnut mushroom (*Agrocybe aegerita*) production: interactions between dietary fiber, physicochemical characteristics, and glycemic load. *Journal Agricultural and Food Chemistry*, 60(17), 4396–4401. <https://doi.org/10.1021/jf3008635>
- Brennan, M. A., Menard, C., Roudaut, G., & Brennan, C. S. (2012b). Amaranth, millet and buckwheat flours affect the physical properties of extruded breakfast cereals and modulates their potential glycaemic impact. *Starch - Stärke*, 64(5), 392–398. <https://doi.org/10.1002/star.201100150>
- Brennan, M. A., Merts, I., Monroe, J., Woolnough, J., & Brennan, C. S. (2008). Impact of guar and wheat bran on the physical and nutritional quality of extruded breakfast cereals. *Starch - Stärke*, 60(5), 248–256. <https://doi.org/10.1002/star.200700698>
- Burešová, I., Tokár, M., Mareček, J., Hřivna, L., Faměra, O., & Šottníková, V. (2017). The comparison of the effect of added amaranth, buckwheat, chickpea, corn, millet and quinoa flour on rice dough rheological characteristics, textural and sensory

- quality of bread. *Journal of Cereal Science*, 75, 158–164. <https://doi.org/10.1016/j.jcs.2017.04.004>
- Burris, J., Shikany, J. M., Rietkerk, W., & Woolf, K. (2018). A low glycemic index and glycemic load diet decreases insulin-like growth factor-1 among adults with moderate and severe acne: a short-duration, 2-week randomized controlled trial. *Journal of the Academy of Nutrition and Dietetics*, 2, 1–12. <https://doi.org/10.1016/j.jand.2018.02.009>
- Bustos, M. C., Perez, G. T., & Leon, A. E. (2015). Structure and quality of pasta enriched with functional ingredients. *Royal Society of Chemistry Advances*, 5(39), 30780–30792. <https://doi.org/10.1039/C4RA11857J>
- Bustos, M. C., Perez, G. T., & León, A. E. (2011). Sensory and nutritional attributes of fibre-enriched pasta. *LWT - Food Science and Technology*, 44(6), 1429–1434. <https://doi.org/10.1016/j.lwt.2011.02.002>
- Cai, L., Wu, X., Zhang, Y., Li, X., Ma, S., & Li, J. (2015). Purification and characterization of three antioxidant peptides from protein hydrolysate of grass carp (*Ctenopharyngodon idella*) skin. *Journal of Functional Foods*, 16, 234–242. <https://doi.org/10.1016/j.jff.2015.04.042>
- Carbonaro, M., Maselli, P., & Nucara, A. (2012). Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: A Fourier transform infrared (FT-IR) spectroscopic study. *Amino Acids*, 43(2), 911–921. <https://doi.org/10.1007/s00726-011-1151-4>
- Cardenas-Hernandez, A., Beta, T., Loarca-Pina, G., Castano-Tostado, E., Nieto-Barrera, J. O., & Mendoza, S. (2016). Improved functional properties of pasta: Enrichment with amaranth seed flour and dried amaranth leaves. *Journal of Cereal Science*, 72, 84–90. <https://doi.org/10.1016/j.jcs.2016.09.014>
- Cascant, M. M., Breil, C., Fabiano-Tixier, A. S., Chemat, F., Garrigues, S., & de la Guardia, M. (2018). Determination of fatty acids and lipid classes in salmon oil by near infrared spectroscopy. *Food Chemistry*, 239, 865–871. <https://doi.org/10.1016/j.foodchem.2017.06.158>
- Casiraghi, M. C., Brighenti, F., Pellegrini, N., Leopardi, E., & Testolin, G. (1993). Effect of processing on rice starch digestibility evaluated by *in vivo* and *in vitro* methods. *Journal of Cereal Science*, 17, 14–156. <https://doi.org/10.1006/jcrs.1993.1015>

- Chalamaiah, M., Dinesh Kumar, B., Hemalatha, R., & Jyothirmayi, T. (2012). Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chemistry*, 135(4), 3020–3038. <https://doi.org/10.1016/j.foodchem.2012.06.100>
- Chang, H. C., & Wu, L. C. (2008). Texture and quality properties of Chinese fresh egg noodles formulated with green seaweed (*Monostroma nitidum*) powder. *Journal of Food Science*, 73(8), 398–404. <https://doi.org/10.1111/j.1750-3841.2008.00912.x>
- Chen, D., Wan, S. B., Yang, H., Yuan, J., Chan, T. H., & Dou, Q. P. (2011). EGCG, green tea polyphenols and their synthetic analogs and prodrugs for human cancer prevention and treatment. *Advances in Clinical Chemistry*, 53(3), 155–177. <https://doi.org/10.1016/B978-0-12-385855-9.00007-2>
- Chen, He, X., Zhang, B., Fu, X., Li, L., & Huang, Q. (2018a). Structure, physicochemical and *in vitro* digestion properties of ternary blends containing swollen maize starch, maize oil and zein protein. *Food Hydrocolloids*, 76, 88–95. <https://doi.org/10.1016/j.foodhyd.2017.04.025>
- Chen, L., Tian, Y., Sun, B., Cai, C., Ma, R., & Jin, Z. (2018b). Measurement and characterization of external oil in the fried waxy maize starch granules using ATR-FTIR and XRD. *Food Chemistry*, 242(4), 131–138. <https://doi.org/10.1016/j.foodchem.2017.09.016>
- Chen, N., Zhao, Q., Sun, W., & Zhao, M. (2013). Effects of malondialdehyde modification on the *in vitro* digestibility of soy protein isolate. *Journal of Agricultural and Food Chemistry*, 61(49), 12139–12145. <https://doi.org/10.1021/jf404099y>
- Chen, X., He, X. W., Zhang, B., Fu, X., Jane, J. lin, & Huang, Q. (2017a). Effects of adding corn oil and soy protein to corn starch on the physicochemical and digestive properties of the starch. *International Journal of Biological Macromolecules*, 104, 481–486. <https://doi.org/10.1016/j.ijbiomac.2017.06.024>
- Chen, Y., Yang, Q., Xu, X., Qi, L., Dong, Z., Luo, Z., ... Peng, X. (2017b). Structural changes of waxy and normal maize starches modified by heat moisture treatment and their relationship with starch digestibility. *Carbohydrate Polymers*, 177(9), 232–240. <https://doi.org/10.1016/j.carbpol.2017.08.121>
- Cheng, L., Feng, T., Zhang, B., Zhu, X., Hamaker, B., Zhang, H., & Campanella, O. (2018).

- A molecular dynamics simulation study on the conformational stability of amylose-linoleic acid complex in water. *Carbohydrate Polymers*, 196(January), 56–65. <https://doi.org/10.1016/j.carbpol.2018.04.102>
- Chiasson, J. L., Josse, R. G., Gomis, R., Hanefeld, M., Karasik, A., & Laakso, M. (2002). Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *The Lancet*, 359(9323), 2072–2077. [https://doi.org/10.1016/S0140-6736\(02\)08905-5](https://doi.org/10.1016/S0140-6736(02)08905-5)
- Chillo, S., Laverse, J., Falcone, P. M., & Del Nobile, M. A. (2008). Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. *Journal of Food Engineering*, 84(1), 101–107. <https://doi.org/10.1016/j.jfoodeng.2007.04.022>
- Chillo, S., Monro, J. A., Mishra, S., & Henry, C. J. (2010). Effect of incorporating legume flour into semolina spaghetti on its cooking quality and glycaemic impact measured *in vitro*. *International Journal of Food Science and Nutrition*, 61(2), 149–160. <https://doi.org/10.3109/09637480903476423>
- Chin, C. K., Huda, N., & Yang, T. A. (2012). Incorporation of surimi powder in wet yellow noodles and its effects on the physicochemical and sensory properties. *International Food Research Journal*, 19(2), 701–707.
- Cho, K. Y., & Rizvi, S. S. H. (2010). New generation of healthy snack food by supercritical fluid extrusion. *Journal of Food Processing and Preservation*, 34(2), 192–218. <https://doi.org/10.1111/j.1745-4549.2009.00372.x>
- Chung, H. J., Liu, Q., Hoover, R., Warkentin, T. D., & Vandenberg, B. (2008). *In vitro* starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food Chemistry*, 111(2), 316–321. <https://doi.org/10.1016/j.foodchem.2008.03.062>
- Chung, H. J., Shin, D. H., & Lim, S. T. (2008). *In vitro* starch digestibility and estimated glycemic index of chemically modified corn starches. *Food Research International*, 41(6), 579–585. <https://doi.org/10.1016/j.foodres.2008.04.006>
- Chung, S. Y., & Champagne, E. T. (2009). Reducing the allergenic capacity of peanut extracts and liquid peanut butter by phenolic compounds. *Food Chemistry*, 115(4), 1345–1349. <https://doi.org/10.1016/j.foodchem.2009.01.052>
- Cleary, L., & Brennan, C. (2006). The influence of a (1 → 3)(1 → 4)-β-D-glucan rich

- fraction from barley on the physico-chemical properties and *in vitro* reducing sugars release of durum wheat pasta. *International Journal of Food Science and Technology*, 41(8), 910–918. <https://doi.org/10.1111/j.1365-2621.2005.01141.x>
- Coda, R., Varis, J., Verni, M., Rizzello, C. G., & Katina, K. (2017). Improvement of the protein quality of wheat bread through faba bean sourdough addition. *LWT - Food Science and Technology*, 82, 296–302. <https://doi.org/10.1016/j.lwt.2017.04.062>
- Coelho, M. S., & Salas-Mellado, M. M. (2015). Effects of substituting chia (*Salvia hispanica* L.) flour or seeds for wheat flour on the quality of the bread. *LWT - Food Science and Technology*, 60(2), 729–736. <https://doi.org/10.1016/j.lwt.2014.10.033>
- Connor, W. E. (2000). Importance of n-3 fatty acids in health and disease. *American Journal of Clinical Nutrition*, 71(3), 1–5.
- Crowe, T. C., Seligman, S. A., & Copeland, L. (2000). Inhibition of enzymic digestion of amylose by free fatty acids *in vitro* contributes to resistant starch formation. *The Journal of Nutrition*, 130(8), 2006–2015. <https://doi.org/10.1093/jn/130.8.2006>
- Czubinski, J., & Dwiecki, K. (2017). A review of methods used for investigation of protein–phenolic compound interactions. *International Journal of Food Science and Technology*, 52(3), 573–585. <https://doi.org/10.1111/ijfs.13339>
- Dai, Z. L. (2011). Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. *Frontiers in Bioscience*, 16(1), 1768. <https://doi.org/10.2741/3820>
- Dalton, A., Wolmarans, P., Witthuhn, R. C., Stuijvenberg, M. E., Swanevelder, S. A., & Smuts, C. M. (2009). A randomised control trial in schoolchildren showed improvement in cognitive function after consuming a bread spread, containing fish flour from a marine source. *Prostaglandins Leukotrienes and Essential Fatty Acids*, 80(2–3), 143–149. <https://doi.org/10.1016/j.plefa.2008.12.006>
- de la Peña, E., & Manthey, F. A. (2014). Ingredient composition and pasta: Water cooking ratio affect cooking properties of nontraditional spaghetti. *International Journal of Food Science and Technology*, 49(10), 2323–2330. <https://doi.org/10.1111/ijfs.12549>
- De Pilli, T., Derossi, A., Talja, R. A., Jouppila, K., & Severini, C. (2011). Study of starch-lipid complexes in model system and real food produced using extrusion-cooking technology. *Innovative Food Science and Emerging Technologies*, 12(4), 610–616.

<https://doi.org/10.1016/j.ifset.2011.07.011>

- Deng, Y., Luo, Y., Wang, Y., & Zhao, Y. (2015). Effect of different drying methods on the myosin structure, amino acid composition, protein digestibility and volatile profile of squid fillets. *Food Chemistry*, 171, 168–176. <https://doi.org/10.1016/j.foodchem.2014.09.002>
- Desai, A., Brennan, M. A., & Brennan, C. S. (2018). The effect of semolina replacement with protein powder from fish (*Pseudophycis bachus*) on the physicochemical characteristics of pasta. *LWT - Food Science and Technology*, 89(6), 52–57. <https://doi.org/10.1016/j.lwt.2017.10.023>
- Dewettinck, K., Van Bockstaele, F., Kühne, B., Van de Walle, D., Courtens, T. M., & Gellynck, X. (2008). Nutritional value of bread: Influence of processing, food interaction and consumer perception. *Journal of Cereal Science*, 48(2), 243–257. <https://doi.org/10.1016/j.jcs.2008.01.003>
- Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Relationship between granule size and *in vitro* digestibility of maize and potato starches. *Carbohydrate Polymers*, 82(2), 480–488. <https://doi.org/10.1016/j.carbpol.2010.05.018>
- Djeukeu, W. A., Gouado, I., Leng, M. S., Vijaykrishnaraj, M., & Prabhasankar, P. (2017). Effect of dried yam flour (*Dioscorea schimperiana*) on cooking quality, digestibility profile and antioxidant potential of wheat based pasta. *Journal of Food Measurement and Characterization*, 11(3), 1421–1429. <https://doi.org/10.1007/s11694-017-9521-6>
- Dziki, D., Rozylo, R., Gawlik-Dziki, U., & Swieca, M. (2014). Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends in Food Science and Technology*, 40(1), 48–61. <https://doi.org/10.1016/j.tifs.2014.07.010>
- Edelmann, A., Diewok, J., Schuster, K. C., & Lendl, B. (2001). Rapid method for the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. *Journal of Agricultural and Food Chemistry*, 49(3), 1139–1145. <https://doi.org/10.1021/jf001196p>
- Egerton, S., Culloty, S., Whooley, J., Stanton, C., & Ross, R. P. (2018). Characterization of protein hydrolysates from blue whiting (*Micromesistius poutassou*) and their application in beverage fortification. *Food Chemistry*, 245(10), 698–706.

- <https://doi.org/10.1016/j.foodchem.2017.10.107>
- El-Beltagi, H. S., El-Senousi, N. A., Ali, Z. A., & Omran, A. A. (2017). The impact of using chickpea flour and dried carp fish powder on pizza quality. *PLoS ONE*, 12(9), 1–15. <https://doi.org/10.1371/journal.pone.0183657>
- El-Khayat, G. H., Samaan, J., Manthey, F. A., Fuller, M. P., & Brennan, C. S. (2006). Durum wheat quality I: Some physical and chemical characteristics of Syrian durum wheat genotypes. *International Journal of Food Science and Technology*, 41(2), 22–29. <https://doi.org/10.1111/j.1365-2621.2006.01245.x>
- European Food Safety Authority (EFSA). (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *European Food Safety Authority Journal*, 8(3), 1461–1471. <https://doi.org/10.2903/j.efsa.2010.1461>.
- Evans, C. E. L., Greenwood, D. C., Threapleton, D. E., Gale, C. P., Cleghorn, C. L., & Burley, V. J. (2017). Glycemic index, glycemic load, and blood pressure : a systematic review and meta-analysis of randomized controlled trials 1. *The American Journal of Clinical Nutrition*, 2(1), 1176–1191. <https://doi.org/10.3945/ajcn.116.143685>. One-third
- Fagundes, G. ., Rocha, M., & Salas-Mellado, M. . (2018). Improvement of protein content and effect on technological properties of wheat. *Food Research*, 2(3), 221–227.
- Fan, L., Ma, S., Wang, X., & Zheng, X. (2016). Improvement of Chinese noodle quality by supplementation with arabinoxylans from wheat bran. *International Journal of Food Science and Technology*, 51(3), 602–608. <https://doi.org/10.1111/ijfs.13042>
- FAO/WHO. (2010). *Report of the joint FAO/WHO expert consultation on the risks and benefits of fish consumption*. *FAO Fisheries and Aquaculture Report* ,978 (2), 11-63.
- FAO. (2013). *Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Cosultation*, 92, 1-79 Retrieved from [http:// www.nutrinform.com/biblioteca/libros_digitales/fao_protein_quality.pdf](http://www.nutrinform.com/biblioteca/libros_digitales/fao_protein_quality.pdf)
- FAO (Food & Agriculture Organisation). (2016). *Fishery and Aquaculture Statistics Statistiques*. *FAO*, 1-230. <https://doi.org/10.5860/CHOICE.50-5350>
- Fares, C., Platani, C., Baiano, A., & Menga, V. (2010). Effect of processing and cooking

- on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat. *Food Chemistry*, 119(3), 1023–1029. <https://doi.org/10.1016/j.foodchem.2009.08.006>
- Farooq, A. M., Dhital, S., Li, C., Zhang, B., & Huang, Q. (2018). Effects of palm oil on structural and *in vitro* digestion properties of cooked rice starches. *International Journal of Biological Macromolecules*, 107(2), 1080–1085. <https://doi.org/10.1016/j.ijbiomac.2017.09.089>
- Farrow, B., & Evers, B. M. (2002). Inflammation and the development of pancreatic cancer. *Surgical Oncology*, 10(4), 153–169. [https://doi.org/10.1016/S0960-7404\(02\)00015-4](https://doi.org/10.1016/S0960-7404(02)00015-4)
- Ficco, D. B. M., De Simone, V., De Leonardis, A. M., Giovanniello, V., Del Nobile, M. A., Padalino, L., ... De Vita, P. (2016). Use of purple durum wheat to produce naturally functional fresh and dry pasta. *Food Chemistry*, 205, 187–195. <https://doi.org/10.1016/j.foodchem.2016.03.014>
- Filipovic, J., Ivkov, M., Kosutic, M., & Filipovic, V. (2016). Ratio of omega-6/omega-3 fatty acids of spelt and flaxseed pasta and consumer acceptability. *Czech Journal of Food Sciences*, 34(6), 522–529. <https://doi.org/10.17221/384/2015-CJFS>
- Filipović, J., Pezo, L., Filipović, V., Brkljača, J., & Krulj, J. (2015). The effects of ω -3 fatty acids and inulin addition to spelt pasta quality. *LWT - Food Science and Technology*, 63(1), 43–51. <https://doi.org/10.1016/j.lwt.2015.03.082>
- Foschia, M., Peressini, D., Sensidoni, A., Brennan, M. A., & Brennan, C. S. (2015a). How combinations of dietary fibres can affect physicochemical characteristics of pasta. *LWT - Food Science and Technology*, 61(1), 41–46. <https://doi.org/10.1016/j.lwt.2014.11.010>
- Foschia, M., Peressini, D., Sensidoni, A., Brennan, M. A., & Brennan, C. S. (2015b). Synergistic effect of different dietary fibres in pasta on *in vitro* starch digestion? *Food Chemistry*, 172, 245–250. <https://doi.org/10.1016/j.foodchem.2014.09.062>
- Fradique, M., Batista, A. P., Nunes, M. C., Gouveia, L., Bandarra, N. M., & Raymundo, A. (2013). *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA's source. *LWT - Food Science and Technology*, 50(1), 312–319. <https://doi.org/10.1016/j.lwt.2012.05.006>
- Freitas, D., Le Feunteun, S., Panouillé, M., & Souchon, I. (2018). The important role of

- salivary α -amylase in the gastric digestion of wheat bread starch. *Food & Function*, 9(1), 200–208. <https://doi.org/10.1039/C7FO01484H>
- Friedman, M. (1996). Nutritional value of proteins from different food sources. a review. *Journal of Agricultural and Food Chemistry*, 44(1), 6–29. <https://doi.org/10.1021/jf9400167>
- Fu, B. X. (2008). Asian noodles: History, classification, raw materials, and processing. *Food Research International*, 41(9), 888–902. <https://doi.org/10.1016/j.foodres.2007.11.007>
- Fuad, T., & Prabhasankar, P. (2012). Influences of india's local wheat varieties and additives on quality of pasta: wheat species and pasta: wheat species and pasta. *Food and Bioprocess Technology*, 5(5), 1743–1755. <https://doi.org/10.1007/s11947-011-0679-3>
- Gallegos-Infante, J. A., Rocha-Guzman, N. E., Gonzalez-Laredo, R. F., Ochoa-Martínez, L. A., Corzo, N., Bello-Perez, L. A., ... Peralta-Alvarez, L. E. (2010). Quality of spaghetti pasta containing Mexican common bean flour (*Phaseolus vulgaris* L.). *Food Chemistry*, 119(4), 1544–1549. <https://doi.org/10.1016/j.foodchem.2009.09.040>
- Gao, J., Wang, Y., Dong, Z., & Zhou, W. (2018). Structural and mechanical characteristics of bread and their impact on oral processing: a review. *International Journal of Food Science & Technology*, 53(1), 858–872. <https://doi.org/10.1111/ijfs.13671>
- Gawlik-Dziki, U., Dziki, D., Baraniak, B., & Lin, R. (2009). The effect of simulated digestion *in vitro* on bioactivity of wheat bread with Tartary buckwheat flavones addition. *LWT - Food Science and Technology*, 42(1), 137–143. <https://doi.org/10.1016/j.lwt.2008.06.009>
- Gawlik-Dziki, U., Świeca, M., Dziki, D., Baraniak, B., Tomiło, J., & Czyz, J. (2013). Quality and antioxidant properties of breads enriched with dry onion (*Allium cepa* L.) skin. *Food Chemistry*, 138(2–3), 1621–1628. <https://doi.org/10.1016/j.foodchem.2012.09.151>
- Gelencser, T., Gal, V., Hodsagi, M., & Salgo, A. (2008). Evaluation of quality and digestibility characteristics of resistant starch-enriched pasta. *Food and Bioprocess Technology*, 1(2), 171–179. <https://doi.org/10.1007/s11947-007-0040-z>
- Gilani, G. S., Xiao, C. W., & Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and

- on protein quality. *British Journal of Nutrition*, 108(2), 315–332. <https://doi.org/10.1017/S0007114512002371>
- Gilani, S., Tome, D., Moughan, P., & Burlingame, B. (2012). Report of a sub-committee of the 2011 FAO consultation on “ protein quality evaluation in human nutrition ” on : The assessment of amino acid digestibility in foods for humans and including a collation of published ileal amino acid digestibility data for. *Food and Agriculture Organization (FAO) Expert Consultation Report*, 12(2), 58–78. Retrieved from <http://www.fao.org/ag/humannutrition/36216-04a2f02ec02eafd4f457dd2c9851b4c45.pdf>
- Gilbert, J. A., Bendsen, N. T., Tremblay, A., & Astrup, A. (2011). Effect of proteins from different sources on body composition. *Nutrition, Metabolism and Cardiovascular Diseases*, 21(2), 16–31. <https://doi.org/10.1016/j.numecd.2010.12.008>
- Giménez, M. A., Drago, S. R., Bassett, M. N., Lobo, M. O., & Sammán, N. C. (2016). Nutritional improvement of corn pasta-like product with broad bean (*Vicia faba*) and quinoa (*Chenopodium quinoa*). *Food Chemistry*, 199, 150–156. <https://doi.org/10.1016/j.foodchem.2015.11.065>
- Giuberti, G., Gallo, A., Cerioli, C., Fortunati, P., & Masoero, F. (2015). Cooking quality and starch digestibility of gluten free pasta using new bean flour. *Food Chemistry*, 175, 43–49. <https://doi.org/10.1016/j.foodchem.2014.11.127>
- Gnagnarella, P., Gandini, S., Vecchia, C. La, & Maisonneuve, P. (2008). Glycemic index, glycemic load, and cancer risk: a meta-analysis. *American Journal of Clinical Nutrition*, 87(3), 1793–1801.
- Gobbetti, M., & Ganzle, M. (2013). Chemistry of Cereal Grains. *Handbook on Sourdough Biotechnology*, 10, 1-298. <https://doi.org/10.1007/978-1-4614-5425-0>
- Goes, E. S., Souza, M. L. R., Michka, J. M. G., Kimura, K. S., Lara, J. A. F., Delbem, A. C. B., & Gasparino, E. (2016). Fresh pasta enrichment with protein concentrate of tilapia: nutritional and sensory characteristics. *Food Science and Technology*, 36(1), 76–82. <https://doi.org/10.1590/1678-457x.0020>
- Gómez-Guillén, M. C., Borderías, A. J., & Montero, P. (1997). Chemical interactions of nonmuscle proteins in the network of sardine (*Sardina pilchardus*) muscle gels. *LWT - Food Science and Technology*, 30(6), 602–608. <https://doi.org/10.1006/fstl.1997.0239>

- Gopalakrishnan, J., Menon, R., Padmaja, G., Sajeev, M. S., & Moorthy, S. N. (2011). Nutritional and functional characteristics of protein-fortified pasta from sweet potato. *Food and Nutrition Sciences*, 2(9), 944–955. <https://doi.org/10.4236/fns.2011.29129>
- Graça, C., Fradinho, P., Sousa, I., & Raymundo, A. (2018). Impact of *Chlorella vulgaris* on the rheology of wheat flour dough and bread texture. *LWT - Food Science and Technology*, 89(6), 466–474. <https://doi.org/10.1016/j.lwt.2017.11.024>
- Granfeldt, Y., Bjorck, I., Drews, A., & Yovar, J. (1992). An *in vitro* procedure based on chewing to predict metabolic response to starch in cereal and legume products. *European Journal of Clinical Nutrition*, 46, 649–660
- Graziano, J. (2014). *The state of world fisheries and aquaculture. Food and Agriculture Organization of the United Nations*, 12, 1–258. <https://doi.org/92-5-105177-1>
- Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*, 103(2), 137–149. <https://doi.org/10.1016/j.diabres.2013.11.002>
- Gull, A., Prasad, K., & Kumar, P. (2015). Effect of millet flours and carrot pomace on cooking qualities, color and texture of developed pasta. *LWT - Food Science and Technology*, 63(1), 470–474. <https://doi.org/10.1016/j.lwt.2015.03.008>
- Gunenc, A., Kong, L., Elias, R. J., & Ziegler, G. R. (2018). Inclusion complex formation between high amylose corn starch and alkylresorcinols from rye bran. *Food Chemistry*, 259(4), 1–6. <https://doi.org/10.1016/j.foodchem.2018.02.149>
- Hager, A. S., Czerny, M., Bez, J., Zannini, E., & Arendt, E. K. (2013). Starch properties, *in vitro* digestibility and sensory evaluation of fresh egg pasta produced from oat, teff and wheat flour. *Journal of Cereal Science*, 58(1), 156–163. <https://doi.org/10.1016/j.jcs.2013.03.004>
- Halim, N. R. A., Yusof, H. M., & Sarbon, N. M. (2016). Functional and bioactive properties of fish protein hydrolisates and peptides: A comprehensive review. *Trends in Food Science & Technology*, 51, 24–33. <https://doi.org/10.1016/j.tifs.2016.02.007>
- Hankey, C. (2003). Essentials of human nutrition. *Appetite*, 41(1), 99. [https://doi.org/10.1016/S0195-6663\(03\)00047-3](https://doi.org/10.1016/S0195-6663(03)00047-3)
- Heems, D., Luck, G., Fraudeau, C., & Verette, E. (1998). Fully automated precolumn

- derivatization, on-line dialysis and high- performance liquid chromatographic analysis of amino acids in food, beverages and feedstuff. *Journal of Chromatography A*, 798(1–2), 9–17. [https://doi.org/10.1016/S0021-9673\(97\)01007-8](https://doi.org/10.1016/S0021-9673(97)01007-8)
- Hibbeln, J. R., Davis, J. M., Steer, C., Emmett, P., Rogers, I., Williams, C., & Golding, J. (2007). Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet*, 369(9561), 578–585. [https://doi.org/10.1016/S0140-6736\(07\)60277-3](https://doi.org/10.1016/S0140-6736(07)60277-3)
- Hossain, A. K. M. M., Brennan, M. A., Mason, S. L., Guo, X., Zeng, X. A., & Brennan, C. S. (2017). The effect of astaxanthin-rich microalgae “*Haematococcus pluvialis*” and wholemeal flours incorporation in improving the physical and functional properties of cookies. *Foods*, 6(8), 57. <https://doi.org/10.3390/foods6080057>
- Hsu, H., & Vavak, D. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science*, 45(5), 1269–1273. <https://doi.org/10.1111/j.1365-2621.1977.tb14476.x>
- Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry*, 50(16), 4437–4444. <https://doi.org/10.1021/jf0201529>
- Huda, N., Li Leng, A., & Xian Yee, C. (2010). Chemical composition, colour and linear expansion properties of Malaysian commercial fish cracker (keropok). *Asian Journal of Food and Agro-Industry*, 3(305), 473–482. Retrieved from www.ajofai.info
- Islas-Rubio, A. R., Calderón de la Barca, A. M., Cabrera-Chávez, F., Cota-Gastélum, A. G., & Beta, T. (2014). Effect of semolina replacement with a raw:popped amaranth flour blend on cooking quality and texture of pasta. *LWT - Food Science and Technology*, 57(1), 217–222. <https://doi.org/10.1016/j.lwt.2014.01.014>
- Jackson, A., Key, T., & Williams, C. (2004). Advice on fish consumption: benefits & risks. *Scientific Committee on Nutrition and Toxicity*, 2(7), 1–165. [https://doi.org/10.1016/S0950-2688\(04\)00011-2](https://doi.org/10.1016/S0950-2688(04)00011-2)
- Jahan Mihan, A. (2017). The role of source of protein in regulation of food intake,

- satiety, body weight and body composition. *Journal of Nutritional Health & Food Engineering*, 6(6), 2–9. <https://doi.org/10.15406/jnhfe.2017.06.00223>
- Jarome, T. J., & Lubin, F. D. (2013). Histone lysine methylation: Critical regulator of memory and behavior. *Reviews in the Neurosciences*, 24(4), 375–387. <https://doi.org/10.1515/revneuro-2013-0008>
- Jeyakumari, A., Rahul Das, M. S., Bindu, J., Joshy, C. G., & Zynudheen, A. A. (2016). Optimisation and comparative study on the addition of shrimp protein hydrolysate and shrimp powder on physicochemical properties of extruded snack. *International Journal of Food Science and Technology*, 51(7), 1578–1585. <https://doi.org/10.1111/ijfs.13127>
- Joehnke, M. S., Rehder, A., Sorensen, S., Bjerregaard, C., Sorensen, J. C., & Markedal, K. E. (2018). *In vitro* digestibility of rapeseed and bovine whey protein mixtures. *Journal of Agricultural and Food Chemistry*, 66(3), 711–719. <https://doi.org/10.1021/acs.jafc.7b04681>
- Josse, A. R., Atkinson, S. A., Tarnopolsky, M. A., & Phillips, S. M. (2011). Increased consumption of dairy foods and protein during diet and exercise induced weight loss promotes fat mass loss and lean mass gain in overweight and obese premenopausal women. *Journal of Nutrition*, 141(9), 1626–1634. <https://doi.org/10.3945/jn.111.141028>
- Kadam, S. U., & Prabhasankar, P. (2010). Marine foods as functional ingredients in bakery and pasta products. *Food Research International*, 43(8), 1975–1980. <https://doi.org/10.1016/j.foodres.2010.06.007>
- Kadam, S. U., & Prabhasankar, P. (2012). Evaluation of cooking, microstructure, texture and sensory quality characteristics of shrimp meat-based pasta. *Journal of Texture Studies*, 43(4), 268–274. <https://doi.org/10.1111/j.1745-4603.2011.00336.x>
- Kalupahana, N. S., Claycombe, K., Newman, S. J., Stewart, T., Siriwardhana, N., Matthan, N., ... Moustaid-Moussa, N. (2010). Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet induced obese mice via modulation of adipose tissue inflammation. *Journal of Nutrition*, 140(11), 1915–1922. <https://doi.org/10.3945/jn.110.125732>
- Kawai, K., Takato, S., Sasaki, T., & Kajiwara, K. (2012). Complex formation, thermal properties, and *in vitro* digestibility of gelatinized potato starch-fatty acid mixtures.

- Food Hydrocolloids*, 27(1), 228–234. <https://doi.org/10.1016/j.foodhyd.2011.07.003>
- Khalili Tilami, S., & Sampels, S. (2018). Nutritional value of fish: lipids, proteins, vitamins, and minerals. *Reviews in Fisheries Science and Aquaculture*, 26(2), 243–253. <https://doi.org/10.1080/23308249.2017.1399104>
- Khan, I., Yousif, A., Johnson, S. K., & Gamlath, S. (2013). Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta. *Food Research International*, 54(1), 578–586. <https://doi.org/10.1016/j.foodres.2013.07.059>
- Khan, I., Yousif, A. M., Johnson, S. K., & Gamlath, S. (2014). Effect of sorghum flour addition on *in vitro* starch digestibility, cooking quality, and consumer acceptability of durum wheat pasta. *Journal of Food Science*, 79(8), 1559–1566. <https://doi.org/10.1111/1750-3841.12542>
- Kim, E. H. J., Petrie, J. R., Motoi, L., Morgenstern, M. P., Sutton, K. H., Mishra, S., & Simmons, L. D. (2008). Effect of structural and physicochemical characteristics of the protein matrix in pasta on *in vitro* starch digestibility. *Food Biophysics*, 3(2), 229–234. <https://doi.org/10.1007/s11483-008-9066-7>
- Kim, S. K., & Mendis, E. (2006). Bioactive compounds from marine processing byproducts - A review. *Food Research International*, 39(4), 383–393. <https://doi.org/10.1016/j.foodres.2005.10.010>
- Kinnula, V. L., & Crapo, J. D. (2004). Superoxide dismutases in malignant cells and human tumors. *Free Radical Biology and Medicine*, 36(6), 718–744. <https://doi.org/10.1016/j.freeradbiomed.2003.12.010>
- Kolanowski, W., & Laufenberg, G. (2006). Enrichment of food products with polyunsaturated fatty acids by fish oil addition. *European Food Research and Technology*, 222(3–4), 472–477. <https://doi.org/10.1007/s00217-005-0089-8>
- Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, 106(21), 2747–2757. <https://doi.org/10.1161/01.CIR.0000038493.65177.94>
- Krishnan, J. G., Menon, R., Padmaja, G., Sajeew, M. S., & Moorthy, S. N. (2012). Evaluation of nutritional and physico-mechanical characteristics of dietary fiber-enriched sweet potato pasta. *European Food Research and Technology*, 234(3),

- 467–476. <https://doi.org/10.1007/s00217-011-1657-8>
- Kumar, L., Brennan, M. A., Mason, S. L., Zheng, H., & Brennan, C. S. (2017). Rheological, pasting and microstructural studies of dairy protein–starch interactions and their application in extrusion-based products: A review. *Starch/Staerke*, 69(1–2), 1–11. <https://doi.org/10.1002/star.201600273>
- Kumar, L., Brennan, M., Zheng, H., & Brennan, C. (2018). The effects of dairy ingredients on the pasting, textural, rheological, freeze-thaw properties and swelling behaviour of oat starch. *Food Chemistry*, 245(9), 518–524. <https://doi.org/10.1016/j.foodchem.2017.10.125>
- Kuo, J. M., Yeh, D. B., & Pan, B. S. (1999). Rapid photometric assay evaluating antioxidative activity in edible plant material. *Journal of Agricultural and Food Chemistry*, 47(8), 3206–3209. <https://doi.org/10.1021/jf981351o>
- Labuckas, D. O., Maestri, D. M., Perelló, M., Martínez, M. L., & Lamarque, A. L. (2008). Phenolics from walnut (*Juglans regia* L.) kernels: Antioxidant activity and interactions with proteins. *Food Chemistry*, 107(2), 607–612. <https://doi.org/10.1016/j.foodchem.2007.08.051>
- Lager, S., & Powell, T. L. (2012). Regulation of nutrient transport across the placenta. *Journal of Pregnancy*, 51(2), 1–14. <https://doi.org/10.1155/2012/179827>
- Lakshmi Devi, N., Aparna, K., & Kalpana, K. (2013). Utilization of fish mince in formulation and development of pasta products. *International Food Research Journal*, 20(1), 219–224.
- Laleg, K., Barron, C., & Santé-Ihoutellier, V. (2016). Function Protein enriched pasta : structure and digestibility, 7(2), 1196–1207. <https://doi.org/10.1039/c5fo01231g>
- Laleg, K., Barron, C., Sante-Lhoutellier, V., Walrand, S., & Micard, V. (2016). Protein enriched pasta: structure and digestibility of its protein network. *Food and Function*, 7(2), 1196–1207. <https://doi.org/10.1039/c5fo01231g>
- Lamacchia, C., Baiano, A., Lamparelli, S., Padalino, L., La Notte, E., & Luccia, A. D. (2010). Study on the interactions between soy and semolina proteins during pasta making. *Food Research International*, 43(4), 1049–1056. <https://doi.org/10.1016/j.foodres.2010.01.016>
- Larrosa, V., Lorenzo, G., Zaritzky, N., & Califano, A. (2016). Improvement of the texture and quality of cooked gluten-free pasta. *LWT - Food Science and Technology*, 70,

- 96–103. <https://doi.org/10.1016/j.lwt.2016.02.039>
- Latner, J., & Schwartz, M. (1999). The effects of a high carbohydrate, high protein or balanced lunch upon later food intake and hunger ratings. *Appetite*, 22, 119–128.
- Lau, E., Zhou, W., & Henry, C. J. (2016). Effect of fat type in baked bread on amylose-lipid complex formation and glycaemic response. *British Journal of Nutrition*, 115(4), 2122–2129. <https://doi.org/10.1017/S0007114516001458>
- Layman, D. K., Evans, E., Baum, J. I., Seyler, J., Erickson, D. J., & Boileau, R. A. (2005). Human nutrition and metabolism dietary protein and exercise have additive effects on body composition during weight loss in adult women. *The Journal of Nutrition*, 135(10), 1903–1910.
- Lazaridou, A., & Biliaderis, C. G. (2007). Molecular aspects of cereal β -glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science*, 46(2), 101–118. <https://doi.org/10.1016/j.jcs.2007.05.003>
- Leser, S. (2013). The 2013 FAO report on dietary protein quality evaluation in human nutrition: Recommendations and implications. *Nutrition Bulletin*, 38(4), 421–428. <https://doi.org/10.1111/nbu.12063>
- Li, M., Zhu, K. X., Guo, X. N., Brijs, K., & Zhou, H. M. (2014). Natural additives in wheat-based pasta and noodle products: Opportunities for enhanced nutritional and functional properties. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 347–357. <https://doi.org/10.1111/1541-4337.12066>
- Li, W., Pickard, M. D., & Beta, T. (2007). Evaluation of antioxidant activity and electronic taste and aroma properties of antho-beers from purple wheat grain. *Journal of Agricultural and Food Chemistry*, 55(22), 8958–8966. <https://doi.org/10.1021/jf071715p>
- Li, X., Gao, X., Lu, J., Mao, X., Wang, Y., Feng, D., ... Gao, W. (2019). Complex formation, physicochemical properties of different concentration of palmitic acid yam (*Dioscorea pposita* Thunb.) starch preparation mixtures. *LWT - Food Science and Technology*, 101(11), 130–137. <https://doi.org/10.1016/j.lwt.2018.11.032>
- Li, Z., Wang, L., Chen, Z., Yu, Q., & Feng, W. (2018). Impact of protein content on processing and texture properties of waxy rice flour and glutinous dumpling. *Journal of Cereal Science*, 81, 30–36. <https://doi.org/10.1016/j.jcs.2018.03.005>

- Lin, J., & Zhou, W. (2018). Role of quercetin in the physicochemical properties, antioxidant and antiglycation activities of bread. *Journal of Functional Foods*, 40(5), 299–306. <https://doi.org/10.1016/j.jff.2017.11.018>
- Liu, P., Wang, R., Kang, X., Cui, B., & Yu, B. (2018a). Effects of ultrasonic treatment on amylose-lipid complex formation and properties of sweet potato starch-based films. *Ultrasonics Sonochemistry*, 44(5), 215–222. <https://doi.org/10.1016/j.ultsonch.2018.02.029>
- Liu, T., Hamid, N., Kantono, K., Pereira, L., Farouk, M. M., & Knowles, S. O. (2016). Effects of meat addition on pasta structure, nutrition and *in vitro* digestibility. *Food Chemistry*, 213, 108–114. <https://doi.org/10.1016/j.foodchem.2016.06.058>
- Liu, W., Brennan, M., Serventi, L., & Brennan, C. (2017a). Buckwheat flour inclusion in Chinese steamed bread: potential reduction in glycemic response and effects on dough quality. *European Food Research and Technology*, 243(5), 727–734. <https://doi.org/10.1007/s00217-016-2786-x>
- Liu, W., Brennan, M., Serventi, L., & Brennan, C. (2017b). Effect of wheat bran on dough rheology and final quality of Chinese steamed bread. *Cereal Chemistry*, 94(3), 581–587. <https://doi.org/10.1094/CCHEM-09-16-0234-R>
- Liu, X., Zhao, J., Zhang, X., Li, Y., Zhao, J., Li, T., ... Qiao, L. (2018b). Enrichment of soybean dietary fiber and protein fortified rice grain by dry flour extrusion cooking: the physicochemical, pasting, taste, palatability, cooking and starch digestibility properties. *Royal Society of Chemistry Advances*, 8(47), 26682–26690. <https://doi.org/10.1039/C8RA01781F>
- Lorusso, A., Verni, M., Montemurro, M., Coda, R., Gobbetti, M., & Rizzello, C. G. (2017). Use of fermented quinoa flour for pasta making and evaluation of the technological and nutritional features. *LWT - Food Science and Technology*, 78, 215–221. <https://doi.org/10.1016/j.lwt.2016.12.046>
- Lu, Brennan, M. A., Serventi, L., Mason, S., & Brennan, C. S. (2016). How the inclusion of mushroom powder can affect the physicochemical characteristics of pasta. *International Journal of Food Science and Technology*, 51(11), 2433–2439. <https://doi.org/10.1111/ijfs.13246>
- Lu, Q., Guo, S., & Zhang, S. (2009). Effects of flour free lipids on textural and cooking qualities of Chinese noodles. *Food Research International*, 42(2), 226–230.

- <https://doi.org/10.1016/j.foodres.2008.11.007>
- Lu, X., Brennan, M. A., Serventi, L., & Brennan, C. S. (2018a). Incorporation of mushroom powder into bread dough-effects on dough rheology and bread properties. *Cereal Chemistry*, 12(1), 418–427. <https://doi.org/10.1002/cche.10043>
- Lu, X., Brennan, M. A., Serventi, L., Liu, J., Guan, W., & Brennan, C. S. (2018b). Addition of mushroom powder to pasta enhances the antioxidant content and modulates the predictive glycaemic response of pasta. *Food Chemistry*, 264(4), 199–209. <https://doi.org/10.1016/j.foodchem.2018.04.130>
- Lu, Z. H., Donner, E., & Liu, Q. (2018). Effect of roasted pea flour/starch and encapsulated pea starch incorporation on the *in vitro* starch digestibility of pea breads. *Food Chemistry*, 245(9), 71–78. <https://doi.org/10.1016/j.foodchem.2017.10.037>
- Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., & Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics*, 103(3), 26–36. <https://doi.org/10.1542/peds.103.3.e26>
- Lund, E. K. (2013). Health benefits of seafood; Is it just the fatty acids? *Food Chemistry*, 140(3), 413–420. <https://doi.org/10.1016/j.foodchem.2013.01.034>
- Luque de Castro, M. D., & Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea. *Journal of Chromatography A*, 1217(16), 2383–2389. <https://doi.org/10.1016/j.chroma.2009.11.027>
- Ma, Y., Zhou, M., & Huang, H. (2014). Changes of heat-treated soymilks in bioactive compounds and their antioxidant activities under *in vitro* gastrointestinal digestion. *European Food Research and Technology*, 239(4), 637–652. <https://doi.org/10.1007/s00217-014-2260-6>
- Ma, Z., Boye, J. I., & Hu, X. (2017). *In vitro* digestibility, protein composition and techno-functional properties of Saskatchewan grown yellow field peas (*Pisum sativum* L.) as affected by processing. *Food Research International*, 92, 64–78. <https://doi.org/10.1016/j.foodres.2016.12.012>
- Madani, Z., Malaisse, W. J., & Ait-Yahia, D. (2015). A comparison between the impact of two types of dietary protein on brain glucose concentrations and oxidative stress in high fructose-induced metabolic syndrome rats. *Biomedical Reports*, 3(5), 731–735. <https://doi.org/10.3892/br.2015.498>

- Maehre, H.K., Dalhelm, L., Edvinsen, G.K., Elvevoll, E.O., & Jensen, I.J. (2018). Protein determination-method matters. *Foods*, 7 (5), 1-12.
- Majumdar, R. K., & Singh, R. K. R. (2014). The effect of extrusion conditions on the physicochemical properties and sensory characteristics of fish-based expanded snacks. *Journal of Food Processing and Preservation*, 38(3), 864–879. <https://doi.org/10.1111/jfpp.12041>
- Marik, P. E., & Varon, J. (2009). Omega-3 dietary supplements and the risk of cardiovascular events: A systematic review. *Clinical Cardiology*, 32(7), 365–372. <https://doi.org/10.1002/clc.20604>
- Marinangeli, C. P. F., & House, J. D. (2017). Potential impact of the digestible indispensable amino acid score as a measure of protein quality on dietary regulations and health. *Nutrition Reviews*, 75(8), 658–667. <https://doi.org/10.1093/nutrit/nux025>
- Marpalle, P., Sonawane, S. K., & Arya, S. S. (2014). Effect of flaxseed flour addition on physicochemical and sensory properties of functional bread. *LWT - Food Science and Technology*, 58(2), 614–619. <https://doi.org/10.1016/j.lwt.2014.04.003>
- Marti, A., Fongaro, L., Rossi, M., Lucisano, M., & Ambrogina Pagani, M. (2011). Quality characteristics of dried pasta enriched with buckwheat flour. *International Journal of Food Science and Technology*, 46(11), 2393–2400. <https://doi.org/10.1111/j.1365-2621.2011.02762.x>
- Martínez, M. L., Marín, M. A., Gili, R. D., Penci, M. C., & Ribotta, P. D. (2017). Effect of defatted almond flour on cooking, chemical and sensorial properties of gluten-free fresh pasta. *International Journal of Food Science and Technology*, 52(10), 2148–2155. <https://doi.org/10.1111/ijfs.13493>
- Matos, J., Lourenço, H. M., Brito, P., Maulvault, A. L., Martins, L. L., & Afonso, C. (2015). Influence of bioaccessibility of total mercury, methyl-mercury and selenium on the risk/benefit associated to the consumption of raw and cooked blue shark (*Prionace glauca*). *Environmental Research*, 143, 123–129. <https://doi.org/10.1016/j.envres.2015.09.015>
- McCleary, M., McNally, M., & Rossiter, P. (2002). Measurement of resistant starch by enzymic digestion in starch and selected plant materials: collaborative study. *Journal of AOAC International*, 85(12), 1103–1111.

- McGuire, S. (2015). The state of food insecurity in the world: meeting the 2015 international hunger targets: taking stock of uneven progress. *Advances in Nutrition*, 15(6), 1–54. <https://doi.org/10.1016/j.advnut.2015.05.001>
- McKnight, J. R., Satterfield, M. C., Jobgen, W. S., Smith, S. B., Spencer, T. E., Meininger, C. J., ... Wu, G. (2010). Beneficial effects of L-arginine on reducing obesity: Potential mechanisms and important implications for human health. *Amino Acids*, 39(2), 349–357. <https://doi.org/10.1007/s00726-010-0598-z>
- Mesa, N. J. E., Alavi, S., Singh, N., Shi, Y. C., Dogan, H., & Sang, Y. (2009). Soy protein-fortified expanded extrudates: Baseline study using normal corn starch. *Journal of Food Engineering*, 90(2), 262–270. <https://doi.org/10.1016/j.jfoodeng.2008.06.032>
- Millward, D. J. (2012). Amino acid scoring patterns for protein quality assessment. *British Journal of Nutrition*, 108(2), 31–43. <https://doi.org/10.1017/S0007114512002462>
- Mirrahimi, A., de Souza, R. J., Chiavaroli, L., Sievenpiper, J. L., Beyene, J., Hanley, A. J., ... Jenkins, D. J. A. (2012). Associations of glycemic index and load with coronary heart disease events: a systematic review and meta-analysis of prospective cohorts. *Journal of the American Heart Association*, 1(5), 1–14. <https://doi.org/10.1161/JAHA.112.000752>
- Mohammed, I., Ahmed, A. R., & Senge, B. (2012). Dough rheology and bread quality of wheat-chickpea flour blends. *Industrial Crops and Products*, 36(1), 196–202. <https://doi.org/10.1016/j.indcrop.2011.09.006>
- Montalbano, A., Tesoriere, L., Diana, P., Barraja, P., Carbone, A., Spanò, V., ... Cirrincione, G. (2016). Quality characteristics and *in vitro* digestibility study of barley flour enriched ditalini pasta. *LWT - Food Science and Technology*, 72, 223–228. <https://doi.org/10.1016/j.lwt.2016.04.042>
- Monteiro, M. L. G., Mársico, E. T., Soares, M. S., Deliza, R., de Oliveira, D. C. R., & Conte-Junior, C. A. (2018). Tilapia-waste flour as a natural nutritional replacer for bread: A consumer perspective. *PLoS ONE*, 13(5), 1–14. <https://doi.org/10.1371/journal.pone.0196665>
- Monteiro, M. L. G., Mársico, E. T., Soares, M. S., Magalhães, A. O., Canto, A. C. V. C. S., Costa-Lima, B. R. C., ... Conte, C. A. (2016). Nutritional profile and chemical stability

- of pasta fortified with tilapia (*Oreochromis niloticus*) flour. *PLoS ONE*, 11(12), 1–17. <https://doi.org/10.1371/journal.pone.0168270>
- Morales, P., Cebadera-Miranda, L., Cámara, R. M., Reis, F. S., Barros, L., Berrios, J. D. J., ... Cámara, M. (2015). Lentil flour formulations to develop new snack-type products by extrusion processing: Phytochemicals and antioxidant capacity. *Journal of Functional Foods*, 19, 537–544. <https://doi.org/10.1016/j.jff.2015.09.044>
- Morens, C., Bos, C., Pueyo, M. E., Benamouzig, R., Gausserès, N., Luengo, C., ... Gaudichon, C. (2003). Increasing habitual protein intake accentuates differences in postprandial dietary nitrogen utilization between protein sources in humans. *The Journal of Nutrition*, 133(5), 2733–2740. <https://doi.org/10.1093/jn/133.9.2733>
- Mori, T. A., Burke, V., Puddey, I. B., Watts, G. F., O’Neal, D. N., Best, J. D., & Beilin, L. J. (2000). Eicosapentaenoic acid and docosahexaenoic acid have differential effects on serum lipids and lipoproteins, LDL-particle size, glucose & insulin, in dyslipidaemic men. *Atherosclerosis*, 151(1), 110–111. [https://doi.org/10.1016/S0021-9150\(00\)80500-6](https://doi.org/10.1016/S0021-9150(00)80500-6)
- Moura, C. M. A., Soares Junior, M. S., Fiorda, F. A., Caliari, M., Vera, R., & Grossmann, M. V. E. (2016). Cooking and texture properties of gluten-free fettuccine processed from defatted flaxseed flour and rice flour. *International Journal of Food Science and Technology*, 51(6), 1495–1501. <https://doi.org/10.1111/ijfs.13097>
- Mullie, P., Koechlin, A., Boniol, M., Autier, P., & Boyle, P. (2016). Relation between breast cancer and high glycemic index or glycemic load: a meta-analysis of prospective cohort studies. *Critical Reviews in Food Science and Nutrition*, 56(1), 152–159. <https://doi.org/10.1080/10408398.2012.718723>
- Mursu, J., Voutilainen, S., Nurmi, T., Tuomainen, T.-P., Kurl, S., & Salonen, J. T. (2008). Flavonoid intake and the risk of ischaemic stroke and CVD mortality in middle-aged finnish men: the kuopio ischaemic heart disease risk factor study. *British Journal of Nutrition*, 100(4), 890–895. <https://doi.org/10.1017/S0007114508945694>
- Nalinanon, S., Benjakul, S., Kishimura, H., & Shahidi, F. (2011). Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna. *Food Chemistry*, 124(4), 1354–1362. <https://doi.org/10.1016/j.foodchem.2010.07.089>
- Nazeer, R. A., & Anila Kulandai, K. (2012). Evaluation of antioxidant activity of muscle

- and skin protein hydrolysates from giant kingfish, *Caranx ignobilis* (Forsskal, 1775). *International Journal of Food Science and Technology*, 47(2), 274–281. <https://doi.org/10.1111/j.1365-2621.2011.02836.x>
- Nikolic, N., Sakac, M., & Mastilovic, J. (2011). Effect of buckwheat flour addition to wheat flour on acylglycerols and fatty acids composition and rheology properties. *LWT - Food Science and Technology*, 44(3), 650–655. <https://doi.org/10.1016/j.lwt.2010.08.017>
- Nilsson, M., Stenberg, M., Frid, A. H., & Holst, J. J. (2004). Glycemia and insulinemia in healthy subjects after lactose- equivalent meals of milk and other food proteins : the role of plasma amino acids and incretins 1 – 3. *The American Journal of Clinical Nutrition*, 80(5), 1246–1253. <https://doi.org/10.1093/ajcn/80.5.1246> [pii]
- Nogueira, A. C., & Steel, C. J. (2018). Protein enrichment of biscuits: a review. *Food Reviews International*, 34(8), 796–809. <https://doi.org/10.1080/87559129.2018.1441299>
- Noni, I., & Pagani, M. A. (2010). Cooking properties and heat damage of dried pasta as influenced by raw material characteristics and processing conditions. *Critical Reviews in Food Science and Nutrition*, 50(5), 465–472. <https://doi.org/10.1080/10408390802437154>
- Nosworthy, M. G., Franczyk, A., Zimoch-Korzycka, A., Appah, P., Utioh, A., Neufeld, J., & House, J. D. (2017). Impact of processing on the protein quality of pinto bean (*Phaseolus vulgaris*) and buckwheat (*Fagopyrum esculentum* Moench) flours and blends, as determined by *in vitro* and *in vivo* methodologies. *Journal of Agricultural and Food Chemistry*, 65(19), 3919–3925. <https://doi.org/10.1021/acs.jafc.7b00697>
- Ohm, J. B., Ross, A. S., Peterson, C. J., & Ong, Y. L. (2008). Relationships of high molecular weight glutenin subunit composition and molecular weight distribution of wheat flour protein with water absorption and color characteristics of noodle dough. *Cereal Chemistry*, 85(2), 123–131. <https://doi.org/10.1094/CCHEM-85-2-0123>
- Oikonomou, N. A., & Krokida, M. K. (2011). Literature data compilation of WAI and WSI of extrudate food products. *International Journal of Food Properties*, 14(1), 199–240. <https://doi.org/10.1080/10942910903160422>
- Okumus, B. N., Tacer-Caba, Z., Kahraman, K., & Nilufer-Erdil, D. (2018). Resistant starch

- type V formation in brown lentil (*Lens culinaris* Medikus) starch with different lipids/fatty acids. *Food Chemistry*, 240(4), 550–558. <https://doi.org/10.1016/j.foodchem.2017.07.157>
- Olivato, J. B., Müller, C. M. O., Carvalho, G. M., Yamashita, F., & Grossmann, M. V. E. (2014). Physical and structural characterisation of starch/polyester blends with tartaric acid. *Materials Science and Engineering C*, 39(1), 35–39. <https://doi.org/10.1016/j.msec.2014.02.020>
- Oliveira, I. S. de, Lourenco, L. F. H., Sousa, C. L., Peixoto Joele, M. R. S., & Ribeiro, S. C. ˜o do A. (2015). Composition of MSM from Brazilian catfish and technological properties of fish flour. *Food Control*, 50, 38–44. <https://doi.org/10.1016/j.foodcont.2014.08.018>
- Oñate Narciso, J., & Brennan, C. (2018). Whey and pea protein fortification of rice starches: effects on protein and starch digestibility and starch pasting properties. *Starch/Staerke*, 70(9–10), 1–6. <https://doi.org/10.1002/star.201700315>
- Ortiz, J., Lemus-Mondaca, R., Vega-Galvez, A., Ah-Hen, K., Puente-Diaz, L., Zura-Bravo, L., & Aubourg, S. (2013). Influence of air-drying temperature on drying kinetics, colour, firmness and biochemical characteristics of Atlantic salmon (*Salmo salar* L.) fillets. *Food Chemistry*, 139(1–4), 162–169. <https://doi.org/10.1016/j.foodchem.2013.01.037>
- Oyeyinka, S. A., Singh, S., Venter, S. L., & Amonsou, E. O. (2017). Effect of lipid types on complexation and some physicochemical properties of bambara groundnut starch. *Starch/Staerke*, 69(3–4), 1–10. <https://doi.org/10.1002/star.201600158>
- Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein-phenolic interactions and associated changes. *Food Research International*, 51(2), 954–970. <https://doi.org/10.1016/j.foodres.2013.02.009>
- Padalino, L., Mastromatteo, M., De Vita, P., Maria Ficco, D. B., & Del Nobile, M. A. (2013). Effects of hydrocolloids on chemical properties and cooking quality of gluten-free spaghetti. *International Journal of Food Science and Technology*, 48(5), 972–983. <https://doi.org/10.1111/ijfs.12049>
- Pal, G. K., Kumar, S. B., Prabhasankar, P., & Suresh, P. V. (2017). Inclusion of poultry based food ingredients in the formulation of noodles and their effects on noodle quality characteristics. *Journal of Food Measurement and Characterization*, 11(3),

- 939–947. <https://doi.org/10.1007/s11694-017-9467-8>
- Palmquist, D. L., & Jenkins, T. C. (2003). Challenges with fats and fatty acid methods. *Journal of Animal Science*, 81(12), 3250–3254. <https://doi.org/10.1021/jf00084a019>
- Pansawat, N., Jangchud, K., Jangchud, A., Wuttijumnong, P., Saalia, F. K., Eitenmiller, R. R., & Phillips, R. D. (2008). Effects of extrusion conditions on secondary extrusion variables and physical properties of fish, rice-based snacks. *LWT - Food Science and Technology*, 41(4), 632–641. <https://doi.org/10.1016/j.lwt.2007.05.010>
- Papes, F., Surpili, M. J., Langone, F., Trigo, J. R., & Arruda, P. (2001). The essential amino acid lysine acts as precursor of glutamate in the mammalian central nervous system. *FEBS Letters*, 488(1–2), 34–38. [https://doi.org/10.1016/S0014-5793\(00\)02401-7](https://doi.org/10.1016/S0014-5793(00)02401-7)
- Parada, J., & Santos, J. L. (2016). Interactions between Starch, Lipids, and Proteins in Foods: Microstructure Control for Glycemic Response Modulation. *Critical Reviews in Food Science and Nutrition*, 56(14), 2362–2369. <https://doi.org/10.1080/10408398.2013.840260>
- Parker, B., Noaks, M., Luscombe, N., & Clifton, P. (2002). High- monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. *Diabetes Care*, 25(3), 425–430. <https://doi.org/10.2337/diacare.25.3.425>
- Parvathy, U., Bindu, J., & Joshy, C. G. (2017). Development and optimization of fish-fortified instant noodles using response surface methodology. *International Journal of Food Science and Technology*, 52(3), 608–616. <https://doi.org/10.1111/ijfs.13313>
- Pasini, G., Simonato, B., Giannattasio, M., Peruffo, A. D. B., Curioni, A., & Agrarie, B. (2001). Modifications of wheat Flour proteins during *in vitro* digestion of bread dough , crumb , and crust : an electrophoretic and immunological study. *Journal of Agricultural and Food Chemistry*, 49(10), 2254–2261.
- Pasqualone, A., Gambacorta, G., Summo, C., Caponio, F., Di Miceli, G., Flagella, Z., ... Lenucci, M. S. (2016). Functional, textural and sensory properties of dry pasta supplemented with lyophilized tomato matrix or with durum wheat bran extracts produced by supercritical carbon dioxide or ultrasound. *Food Chemistry*, 213(7), 545–553. <https://doi.org/10.1016/j.foodchem.2016.07.006>

- Pasqualone, A., Punzi, R., Trani, A., Summo, C., Paradiso, V. M., Caponio, F., & Gambacorta, G. (2017). Enrichment of fresh pasta with antioxidant extracts obtained from artichoke canning by-products by ultrasound-assisted technology and quality characterisation of the end product. *International Journal of Food Science and Technology*, 52(9), 2078–2087. <https://doi.org/10.1111/ijfs.13486>
- Pastor-Cavada, E., Drago, S. R., González, R. J., Juan, R., Pastor, J. E., Alaiz, M., & Vioque, J. (2011). Effects of the addition of wild legumes (*Lathyrus annuus* and *Lathyrus clymenum*) on the physical and nutritional properties of extruded products based on whole corn and brown rice. *Food Chemistry*, 128(4), 961–967. <https://doi.org/10.1016/j.foodchem.2011.03.126>
- Pee, S., & Bloem, M. W. (2009). Current and potential role of specially formulated foods and food supplements for preventing malnutrition among 6 to 23 monthold children and for treating moderate malnutrition among 6 to 59 month old children. *Food and Nutrition Bulletin*, 30(3), S434-63. <https://doi.org/10.1017/CBO9781107415324.004>
- Petitot, M., Abecassis, J., & Micard, V. (2009). Structuring of pasta components during processing: impact on starch and protein digestibility and allergenicity. *Trends in Food Science and Technology*, 20(11–12), 521–532. <https://doi.org/10.1016/j.tifs.2009.06.005>
- Petitot, M., Boyer, L., Minier, C., & Micard, V. (2010). Fortification of pasta with split pea and faba bean flours: Pasta processing and quality evaluation. *Food Research International*, 43(2), 634–641. <https://doi.org/10.1016/j.foodres.2009.07.020>
- Phongthai, S., D’Amico, S., Schoenlechner, R., Homthawornchoo, W., & Rawdkuen, S. (2017). Effects of protein enrichment on the properties of rice flour based gluten-free pasta. *LWT - Food Science and Technology*, 80, 378–385. <https://doi.org/10.1016/j.lwt.2017.02.044>
- Phongthai, S., D’Amico, S., Schoenlechner, R., & Rawdkuen, S. (2016). Comparative study of rice bran protein concentrate and egg albumin on gluten-free bread properties. *Journal of Cereal Science*, 72, 38–45. <https://doi.org/10.1016/j.jcs.2016.09.015>
- Pires, C., Costa, S., Batista, A. P., Nunes, M. C., Raymundo, A., & Batista, I. (2012). Properties of protein powder prepared from Cape hake by-products. *Journal of*

<https://doi.org/10.1016/j.jfoodeng.2011.08.020>

- Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, 97, 55–74. <https://doi.org/10.1016/j.ejmech.2015.04.040>
- Pongpichaiudom, A., & Songsermpong, S. (2018). Evaluation of microstructure and quality characteristics of microwave-dried instant noodles enriched with chicken meat, egg yolk, and seaweed. *Journal of Food Measurement and Characterization*, 12(1), 22–34. <https://doi.org/10.1007/s11694-017-9613-3>
- Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R., ... Miyashita, K. (2009). Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chemistry*, 115(2), 501–508. <https://doi.org/10.1016/j.foodchem.2008.12.047>
- Previtali, M. A., Mastromatteo, M., De Vita, P., Ficco, D. B. M., Conte, A., & Del Nobile, M. A. (2014). Effect of the lentil flour and hydrocolloids on baking characteristics of wholemeal durum wheat bread. *International Journal of Food Science & Technology*, 49(11), 2382–2390. <https://doi.org/10.1111/ijfs.12559>
- Prodpran, T., Benjakul, S., & Phatcharat, S. (2012). Effect of phenolic compounds on protein cross-linking and properties of film from fish myofibrillar protein. *International Journal of Biological Macromolecules*, 51(5), 774–782. <https://doi.org/10.1016/j.ijbiomac.2012.07.010>
- Rahman, T., Hosen, I., Islam, M. M. T., & Shekhar, H. U. (2012). Oxidative stress and human health. *Advances in Bioscience and Biotechnology*, 3(7), 997–1019. <https://doi.org/10.4236/abb.2012.327123>
- Raji, C. A., Erickson, K. I., Lopez, O. L., Kuller, L. H., Gach, H. M., Thompson, P. M., ... Becker, J. T. (2014). Regular fish consumption and age-related brain gray matter loss. *American Journal of Preventive Medicine*, 47(4), 444–451. <https://doi.org/10.1016/j.amepre.2014.05.037>
- Ramya, N. S., Prabhasankar, P., Gowda, Lalitha, R., Modi, V. K., & Bhaskar, N. (2015). Influence of freeze-dried shrimp meat in pasta processing qualities of Indian T. durum wheat. *Journal of Aquatic Food Product Technology*, 24(7), 582–596. <https://doi.org/10.1080/10498850.2013.796581>

- Rawel, H. M., Meidtner, K., & Kroll, J. (2005). Binding of selected phenolic compounds to proteins. *Journal of Agricultural and Food Chemistry*, 53(10), 4228–4235. <https://doi.org/10.1021/jf0480290>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Evans, C. R. (1999). Antioxidant Activity Applying an Improved Abts Radical. *Free Radical Biology & Medicine*, 26(9), 1231–1237.
- Reddy, C. K., Choi, S. M., Lee, D. J., & Lim, S. T. (2018). Complex formation between starch and stearic acid: Effect of enzymatic debranching for starch. *Food Chemistry*, 244, 136–142. <https://doi.org/10.1016/j.foodchem.2017.10.040>
- Ren, X., Chen, J., Molla, M. M., Wang, C., Diao, X., & Shen, Q. (2016). *In vitro* starch digestibility and *in vivo* glycemic response of foxtail millet and its products. *Food & Function*, 1(7), 372–379. <https://doi.org/10.1039/c5fo01074h>
- Reshmi, S. K., Sudha, M. L., & Shashirekha, M. N. (2017). Starch digestibility and predicted glycemic index in the bread fortified with pomelo (*Citrus maxima*) fruit segments. *Food Chemistry*, 237, 957–965. <https://doi.org/10.1016/j.foodchem.2017.05.138>
- Rodríguez De Marco, E., Steffolani, M. E., Martínez, C. S., & León, A. E. (2014). Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. *LWT - Food Science and Technology*, 58(1), 102–108. <https://doi.org/10.1016/j.lwt.2014.02.054>
- Rodríguez De Marco, E., Steffolani, M. E., Martínez, M., & León, A. E. (2018). The use of *Nannochloropsis* sp. as a source of omega-3 fatty acids in dry pasta: chemical, technological and sensory evaluation. *International Journal of Food Science & Technology*, 53(2), 499–507. <https://doi.org/10.1111/ijfs.13609>
- Rosa-Sibakov, N., Heiniö, R. L., Cassan, D., Holopainen-Mantila, U., Micard, V., Lantto, R., & Sozer, N. (2016). Effect of bioprocessing and fractionation on the structural, textural and sensory properties of gluten-free faba bean pasta. *LWT - Food Science and Technology*, 67, 27–36. <https://doi.org/10.1016/j.lwt.2015.11.032>
- Rozylo, R., Hameed Hassoon, W., Gawlik-Dziki, U., Siastala, M., & Dziki, D. (2017). Study on the physical and antioxidant properties of gluten-free bread with brown algae. *CyTA - Journal of Food*, 15(2), 196–203. <https://doi.org/10.1080/19476337.2016.1236839>

- Rustad, T., Storro, I., & Slizyte, R. (2011). Possibilities for the utilisation of marine by-products. *International Journal of Food Science and Technology*, 46(10), 2001–2014. <https://doi.org/10.1111/j.1365-2621.2011.02736.x>
- Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F., & Stanton, C. (2011). Bioactive peptides from muscle sources: Meat and fish. *Nutrients*, 3(9), 765–791. <https://doi.org/10.3390/nu3090765>
- Saini, R. K., & Keum, Y. S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance -A review. *Life Sciences*, 203(5), 255–267. <https://doi.org/10.1016/j.lfs.2018.04.049>
- Sala-Vila, A., Miles, E. A., & Calder, P. C. (2008). Fatty acid composition abnormalities in atopic disease: Evidence explored and role in the disease process examined. *Clinical and Experimental Allergy*, 38(9), 1432–1450. <https://doi.org/10.1111/j.1365-2222.2008.03072.x>
- Samaan, J., El-Khayat, G. H., Manthey, F. A., Fuller, M. P., & Brennan, C. S. (2006). Durum wheat quality: II. The relationship of kernel physicochemical composition to semolina quality and end product utilisation. *International Journal of Food Science and Technology*, 41(SUPPL. 2), 47–55. <https://doi.org/10.1111/j.1365-2621.2006.01313.x>
- Sanders, T. A. B. (2010). The role of fat in the diet - quantity, quality and sustainability. *Nutrition Bulletin*, 35(2), 138–146. <https://doi.org/10.1111/j.1467-3010.2010.01824.x>
- Santana, P., Huda, N., & Yang, T. A. (2015). Physicochemical properties and sensory characteristics of sausage formulated with surimi powder. *Journal of Food Science and Technology*, 52(3), 1507–1515. <https://doi.org/10.1007/s13197-013-1145-1>
- Santos, J. L., Saus, E., Smalley, S. V., Cataldo, L. R., Alberti, G., Parada, J., ... Estivill, X. (2012). Copy number polymorphism of the salivary amylase gene: Implications in human nutrition research. *Journal of Nutrigenetics and Nutrigenomics*, 5(3), 117–131. <https://doi.org/10.1159/000339951>
- Sanz-Penella, J. M., Wronkowska, M., Soral-Smietana, M., & Haros, M. (2013). Effect of whole amaranth flour on bread properties and nutritive value. *LWT - Food Science and Technology*, 50(2), 679–685. <https://doi.org/10.1016/j.lwt.2012.07.031>
- Saris, W. H. M. (2005). DiOGenes : an integrated multidisciplinary approach to the

- obesity problem in Europe. *Nutrition Bulletin*, 30, 188–193.
- Sathivel, S., Bechtel, P. J., Babbitt, J., Prinyawiwatukul, W., Negulescu, I. I., & Reppond, K. D. (2004). Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*) byproducts. *Journal of Agricultural and Food Chemistry*, 52(16), 5040–5046. <https://doi.org/10.1021/jf0351422>
- Sayed Ahmad, B., Talou, T., Straumite, E., Sabovics, M., Kruma, Z., Saad, Z., ... Merah, O. (2018). Protein bread fortification with cumin and caraway seeds and by-product flour. *Foods*, 7(3), 17–28. <https://doi.org/10.3390/foods7030028>
- Schaafsma, G. (2005). The protien digestibility-corrected amino acid score (PDCAAS)- A concept for describing protien quality in foods and food ingredients: A critical review. *Journal of AOAC International*, 88(3), 988–994.
- Schaafsma, G. (2012). Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. *British Journal of Nutrition*, 108(2), 333–336. <https://doi.org/10.1017/S0007114512002541>
- Schakel, S. F., Buzzard, I. M., & Gebhardt, S. E. (1997). Procedures for estimating nutrient values for food composition databases. *Journal of Food Composition and Analysis*, 10(2), 102–114. <https://doi.org/10.1006/jfca.1997.0527>
- Schneedorferová, I., Tomčala, A., & Valterová, I. (2015). Effect of heat treatment on the n-3/n-6 ratio and content of polyunsaturated fatty acids in fish tissues. *Food Chemistry*, 176, 205–211. <https://doi.org/10.1016/j.foodchem.2014.12.058>
- Schwingshackl, L., & Hoffmann, G. (2013). Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: A systematic review and meta-analysis. *Nutrition, Metabolism and Cardiovascular Diseases*, 23(8), 699–706. <https://doi.org/10.1016/j.numecd.2013.04.008>
- Seczyk, Ł., Swieca, M., Dziki, D., Anders, A., & Gawlik-Dziki, U. (2017). Antioxidant, nutritional and functional characteristics of wheat bread enriched with ground flaxseed hulls. *Food Chemistry*, 214, 32–38. <https://doi.org/10.1016/j.foodchem.2016.07.068>
- Seczyk, Ł., Swieca, M., & Gawlik-Dziki, U. (2016a). Effect of carob (*Ceratonia siliqua* L.) flour on the antioxidant potential, nutritional quality, and sensory characteristics

- of fortified durum wheat pasta. *Food Chemistry*, 194, 637–642.
<https://doi.org/10.1016/j.foodchem.2015.08.086>
- Sęczyk, Ł., Świeca, M., Gawlik-Dziki, U., Luty, M., & Czyz, J. (2016b). Effect of fortification with parsley (*Petroselinum crispum* Mill.) leaves on the nutraceutical and nutritional quality of wheat pasta. *Food Chemistry*, 190, 419–428.
<https://doi.org/10.1016/j.foodchem.2015.05.110>
- Serna-Saldivar, S. O., & Abril, R. (2011). Production and nutraceutical properties of breads fortified with DHA- and Omega-3-containing oils. *Flour and Breads and Their Fortification in Health and Disease Prevention*, 12(2), 313–323.
<https://doi.org/10.1016/B978-0-12-380886-8.10029-7>
- Shah, A., Zhang, G., Hamaker, B. R., & Campanella, O. H. (2011). Rheological properties of a soluble self-assembled complex from starch, protein and free fatty acids. *Journal of Food Engineering*, 105(3), 444–452.
<https://doi.org/10.1016/j.jfoodeng.2011.02.048>
- Shaheen, N., Islam, S., Munmun, S., Mohiduzzaman, M., & Longvah, T. (2016). Amino acid profiles and digestible indispensable amino acid scores of proteins from the prioritized key foods in Bangladesh. *Food Chemistry*, 213, 83–89.
<https://doi.org/10.1016/j.foodchem.2016.06.057>
- Shahidi, F., & Ambigaipalan, P. (2018). Omega-3 polyunsaturated fatty acids and their health benefits. *Annual Review of Food Science and Technology*, 9(1), 1603–1637.
<https://doi.org/10.1146/annurev-food-111317-095850>
- Shahidi, F., Liyana-Pathirana, C. M., & Wall, D. S. (2006). Antioxidant activity of white and black sesame seeds and their hull fractions. *Food Chemistry*, 99(3), 478–483.
<https://doi.org/10.1016/j.foodchem.2005.08.009>
- Shaviklo, A. R. (2013). Development of fish protein powder as an ingredient for food applications: a review. *Journal of Food Science and Technology*, 52(2), 648–661.
<https://doi.org/10.1007/s13197-013-1042-7>
- Shaviklo, G. R., Olafsdottir, A., Sveinsdottir, K., Thorkelsson, G., & Rafipour, F. (2011). Quality characteristics and consumer acceptance of a high fish protein puffed corn-fish snack. *Journal of Food Science and Technology*, 48(6), 668–676.
<https://doi.org/10.1007/s13197-010-0191-1>
- Shaviklo, G. R., Thorkelsson, G., Sveinsdottir, K., & Pourreza, F. (2013). Studies on

- processing, consumer survey and storage stability of a ready-to-reconstitute fish cutlet mix. *J Food Sci Technol*, 50(5), 900–908. <https://doi.org/10.1007/s13197-011-0417-x>
- Shaviklo, Thorkelsson, G., Arason, S., Kristinsson, H. G., & Sveinsdottir, K. (2010). The influence of additives and drying methods on quality attributes of fish protein powder made from saithe (*Pollachius virens*). *Journal of the Science of Food and Agriculture*, 90(12), 2133–2143. <https://doi.org/10.1002/jsfa.4062>
- Silva, E. M. M. da, Ascheri, J. L. R., Carvalho, C. W. P. de, Takeiti, C. Y., & Berrios, J. de J. (2014). Physical characteristics of extrudates from corn flour and dehulled carioca bean flour blend. *LWT - Food Science and Technology*, 58(2), 620–626. <https://doi.org/10.1016/j.lwt.2014.03.031>
- Silva, E., Gerritsen, L., Dekker, M., Van der Linden, E., & Scholten, E. (2013). High amounts of broccoli in pasta-like products: nutritional evaluation and sensory acceptability. *Food & Function*, 4(11), 1700–1708. <https://doi.org/10.1039/c3fo00012e>
- Silva, J. C., Rodrigues, S., Feás, X., & Estevinho, L. M. (2012). Antimicrobial activity, phenolic profile and role in the inflammation of propolis. *Food and Chemical Toxicology*, 50(5), 1790–1795. <https://doi.org/10.1016/j.fct.2012.02.097>
- Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: a review. *Trends in Food Science and Technology*, 21(4), 168–180. <https://doi.org/10.1016/j.tifs.2009.12.001>
- Singh, R. K. R., Majumdar, R. K., & Venkateshwarlu, G. (2014). Effect of process conditions on physico-chemical and sensory properties of fish-cereal-based extruded snack-like products. *Journal of Food Processing and Preservation*, 38(1), 68–82. <https://doi.org/10.1111/j.1745-4549.2012.00746.x>
- Singleton, V. L., Orthofer, R., & Lamuela-Ravents, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299(3), 152–178.
- Siriwardhana, N., Kalupahana, N. S., & Moustaid-Moussa, N. (2012). Health benefits of n-3 polyunsaturated fatty acids. eicosapentaenoic acid and docosahexaenoic acid. *Advances in Food and Nutrition Research*, 65, 211-222. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-416003-3.00013-5>

- Smatanová, N., & Lacko-Bartošová, M. (2014). Noodle quality of winter wheat cultivated in sustainable farming systems. *Journal of Central European Agriculture*, 15(2), 84–94. <https://doi.org/10.5513/JCEA01/15.2.1457>
- Sobota, A., Rzedzicki, Z., Zarzycki, P., & Kuzawińska, E. (2015). Application of common wheat bran for the industrial production of high-fibre pasta. *International Journal of Food Science and Technology*, 50(1), 111–119. <https://doi.org/10.1111/ijfs.12641>
- Soon, A. L., Hye, J. K., Ki, C. C., Jong, C. B., Ji, K. P., Jeong, K. S., ... Won, Y. P. (2009). DHA and EPA down-regulate COX-2 expression through suppression of NF-κB activity in LPS-treated human umbilical vein endothelial cells. *Korean Journal of Physiology and Pharmacology*, 13(4), 301–307. <https://doi.org/10.4196/kjpp.2009.13.4.301>
- Sopade, P. A. (2017). Cereal processing and glycaemic response. *International Journal of Food Science and Technology*, 52(1), 22–37. <https://doi.org/10.1111/ijfs.13237>
- Sosulski, F. W., & Imafidon, G. I. (1990). Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. *Journal of Agricultural and Food Chemistry*, 38(6), 1351–1356. <https://doi.org/10.1021/jf00096a011>
- Stevanato, F. B., Cottica, S. M., Petenuci, M. E., Matsushita, M., Desouza, N. E., & Visentainer, J. V. (2010). Evaluation of processing, preservation and chemical and fatty acid composition of Nile Tilapia waste. *Journal of Food Processing and Preservation*, 34, 373–383. <https://doi.org/10.1111/j.1745-4549.2009.00429.x>
- Stillwell, W., & Wassall, S. R. (2003). Docosahexaenoic acid: Membrane properties of a unique fatty acid. *Chemistry and Physics of Lipids*, 126(1), 1–27. [https://doi.org/10.1016/S0009-3084\(03\)00101-4](https://doi.org/10.1016/S0009-3084(03)00101-4)
- Sun-Waterhouse, D., Jin, D., & Waterhouse, G. I. N. (2013). Effect of adding elderberry juice concentrate on the quality attributes, polyphenol contents and antioxidant activity of three fibre-enriched pastas. *Food Research International*, 54(1), 781–789. <https://doi.org/10.1016/j.foodres.2013.08.035>
- Sun, Q. J., Sun, L. L., Xiong, L., & Sun, C. X. (2013). Effect of the addition of stearic acid on starch properties. *Advanced Materials Research*, 655–657, 1996–2000. <https://doi.org/10.4028/www.scientific.net/AMR.655-657.1996>
- Swieca, M., Dziki, D., & Gawlik-Dziki, U. (2017). Starch and protein analysis of wheat bread enriched with phenolics-rich sprouted wheat flour. *Food Chemistry*, 228,

- 643–648. <https://doi.org/10.1016/j.foodchem.2017.02.052>
- Swieca, M., Gawlik-Dziki, U., Dziki, D., Baraniak, B., & Czyz, J. (2013). The influence of protein-flavonoid interactions on protein digestibility *in vitro* and the antioxidant quality of breads enriched with onion skin. *Food Chemistry*, 141(1), 451–458. <https://doi.org/10.1016/j.foodchem.2013.03.048>
- Swieca, M., Gawlik-Dziki, U., Seczyk, Ł., Dziki, D., & Sikora, M. (2018). Interactions of green coffee bean phenolics with wheat bread matrix in a model of simulated *in vitro* digestion. *Food Chemistry*, 258(7), 301–307. <https://doi.org/10.1016/j.foodchem.2018.03.081>
- Swieca, M., Seczyk, Ł., Gawlik-Dziki, U., & Dziki, D. (2014). Bread enriched with quinoa leaves - The influence of protein-phenolics interactions on the nutritional and antioxidant quality. *Food Chemistry*, 162, 54–62. <https://doi.org/10.1016/j.foodchem.2014.04.044>
- Szymanski, K. M., Wheeler, D. C., & Mucci, L. A. (2010). Fish consumption and prostate cancer risk: a review and meta-analysis. *The American Journal of Clinical Nutrition*, 92(5), 1223–33. <https://doi.org/10.3945/ajcn.2010.29530>
- Tacon, A. G. J., & Metian, M. (2013). Fish matters: importance of aquatic foods in human nutrition and global food supply. *Reviews in Fisheries Science*, 21(1), 22–38. <https://doi.org/10.1080/10641262.2012.753405>
- Tacon, A. G. J., & Metian, M. (2018). Food matters: fish, income, and food supply-A comparative analysis. *Reviews in Fisheries Science and Aquaculture*, 26(1), 15–28. <https://doi.org/10.1080/23308249.2017.1328659>
- Takeungwongtrakul, S., Benjakul, S., & Kittikun, Aran, H. (2015). Characteristics and oxidative stability of bread fortified with encapsulated shrimp oil. *Italian Journal of Food Science*, 27(4), 476–486. <https://doi.org/10.14674/1120-1770/ijfs.v380>
- Tan, I., Flanagan, B. M., Halley, P. J., Whittaker, A. K., & Gidley, M. J. (2007). A method for estimating the nature and relative proportions of amorphous, single, and doubled-helical components in starch granules by ¹³C CP/MAS NMR. *Biomacromolecules*, 8(3), 885–891. <https://doi.org/10.1021/bm060988a>
- Tavano, O. L., Neves, V. A., & da Silva Júnior, S. I. (2016). *In vitro* versus *in vivo* protein digestibility techniques for calculating PDCAAS (protein digestibility-corrected amino acid score) applied to chickpea fractions. *Food Research International*, 89,

- 756–763. <https://doi.org/10.1016/j.foodres.2016.10.005>
- Tazart, K., Lamacchia, C., Zaidi, F., & Haros, M. (2016). Nutrient composition and *in vitro* digestibility of fresh pasta enriched with *Vicia faba*. *Journal of Food Composition and Analysis*, 47, 8–15. <https://doi.org/10.1016/j.jfca.2015.12.007>
- Tazart, K., Zaidi, F., Lamacchia, C., & Haros, M. (2015). Effect of durum wheat semolina substitution with broad bean flour (*Vicia faba*) on the Maccheronccini pasta quality. *European Food Research and Technology*, 242(4), 477–485. <https://doi.org/10.1007/s00217-015-2558-z>
- Teixeira, B., Pires, C., Nunes, M. L., & Batista, I. (2016). Effect of *in vitro* gastrointestinal digestion on the antioxidant activity of protein hydrolysates prepared from Cape hake by-products. *International Journal of Food Science and Technology*, 51(12), 2528–2536. <https://doi.org/10.1111/ijfs.13233>
- Thachil, M. T., Chouksey, M. K., & Gudipati, V. (2014). Amylose-lipid complex formation during extrusion cooking: Effect of added lipid type and amylose level on corn-based puffed snacks. *International Journal of Food Science and Technology*, 49(2), 309–316. <https://doi.org/10.1111/ijfs.12333>
- Tieland, M., Borgonjen-Van Den Berg, K. J., Van Loon, L. J. C., & de Groot, L. C. P. G. M. (2015). Dietary protein intake in dutch elderly people: A focus on protein sources. *Nutrients*, 7(12), 9697–9706. <https://doi.org/10.3390/nu7125496>
- Torres, A., Frias, J., Granito, M., & Vidal-Valverde, C. (2006). Germinated *Cajanus cajan* seeds as ingredients in pasta products: Chemical, biological and sensory evaluation. *Food Chemistry*, 101(1), 202–211. <https://doi.org/10.1016/j.foodchem.2006.01.018>
- Turco, I., Bacchetti, T., Bender, C., Zimmermann, B., Oboh, G., & Ferretti, G. (2016). Polyphenol content and glycemic load of pasta enriched with Faba bean flour. *Functional Foods in Health and Disease*, 6(5), 291–305. Retrieved from <http://ffhdj.com/index.php/ffhd/article/view/254>
- Turfani, V., Narducci, V., Durazzo, A., Galli, V., & Carcea, M. (2017). Technological, nutritional and functional properties of wheat bread enriched with lentil or carob flours. *LWT - Food Science and Technology*, 78, 361–366. <https://doi.org/10.1016/j.lwt.2016.12.030>
- FAO,WHO,UNU (2007). Protein and amino acid requirements in human nutrition.

Report of the Joint WHO/FAO/UNU Expert Consultation. WHO Technical Report Series, 935. World Health Organization, Geneva, Switzerland.

- Usydus, Z., Szlinder-Richert, J., & Adamczyk, M. (2009). Protein quality and amino acid profiles of fish products available in Poland. *Food Chemistry*, 112(1), 139–145. <https://doi.org/10.1016/j.foodchem.2008.05.050>
- Utrilla-Coello, R. G., Rodríguez-Huezo, M. E., Carrillo-Navas, H., Hernández-Jaimes, C., Vernon-Carter, E. J., & Alvarez-Ramirez, J. (2014). *In vitro* digestibility, physicochemical, thermal and rheological properties of banana starches. *Carbohydrate Polymers*, 101(1), 154–162. <https://doi.org/10.1016/j.carbpol.2013.09.019>
- Vajragupta, O., Boonchoong, P., & Wongkrajang, Y. (2000). Comparative quantitative structure activity study of radical scavengers. *Bioorganic and Medicinal Chemistry*, 8(11), 2617–2628.
- Vakily, J. M., Seto, K., & Pauly, D. (2012). The marine fisheries environment of Sierra Leone. *Fisheries Centre Research Reports*, 20(4), 102–130.
- Van Den Elsen, L. W. J., Noakes, P. S., Van Der Maarel, M. A., Kremmyda, L. S., Vlachava, M., Diaper, N. D., ... Calder, P. C. (2011). Salmon consumption by pregnant women reduces *ex vivo* umbilical cord endothelial cell activation. *American Journal of Clinical Nutrition*, 94(6), 1418–1425. <https://doi.org/10.3945/ajcn.111.016592>
- Vargas-Torres, A., Osorio-Díaz, P., Islas-Hernández, J. J., Tovar, J., Paredes-López, O., & Bello-Pérez, L. A. (2004). Starch digestibility of five cooked black bean (*Phaseolus vulgaris* L.) varieties. *Journal of Food Composition and Analysis*, 17(5), 605–612. <https://doi.org/10.1016/j.jfca.2003.09.008>
- Venugopal, V. (1992). Mince from low-cost fish species. *Trends in Food Science and Technology*, 3(1), 2–5. [https://doi.org/10.1016/0924-2244\(92\)90102-3](https://doi.org/10.1016/0924-2244(92)90102-3)
- Verardo, V., Ferioli, F., Riciputi, Y., lafelice, G., Marconi, E., & Caboni, M. (2009). Evaluation of lipid oxidation in spaghetti pasta enriched with long chain n–3 polyunsaturated fatty acids under different storage conditions. *Food Chemistry*, 114(2), 472–477. <https://doi.org/10.1016/j.foodchem.2008.09.074>
- Vijaykrishnaraj, M., Bharath Kumar, S., & Prabhasankar, P. (2014). Green mussel (*Perna canaliculus*) as a marine ingredient to enrich gluten free pasta: product quality, microstructure and biofunctional evaluation. *Journal of Food Measurement and*

- Characterization*, 9(1), 76–85. <https://doi.org/10.1007/s11694-014-9212-5>
- Vijaykrishnaraj, M., Roopa, B. S., & Prabhasankar, P. (2016). Preparation of gluten free bread enriched with green mussel (*Perna canaliculus*) protein hydrolysates and characterization of peptides responsible for mussel flavour. *Food Chemistry*, 211, 715–725. <https://doi.org/10.1016/j.foodchem.2016.05.094>
- Villarino, C. B. J., Jayasena, V., Coorey, R., Chakrabarti-Bell, S., Foley, R., Fanning, K., & Johnson, S. K. (2015a). The effects of lupin (*Lupinus angustifolius*) addition to wheat bread on its nutritional, phytochemical and bioactive composition and protein quality. *Food Research International*, 76(1), 58–65. <https://doi.org/10.1016/j.foodres.2014.11.046>
- Villarino, C. B. J., Jayasena, V., Coorey, R., Chakrabarti-Bell, S., & Johnson, S. K. (2015b). The effects of Australian sweet lupin (ASL) variety on physical properties of flours and breads. *LWT - Food Science and Technology*, 60(1), 435–443. <https://doi.org/10.1016/j.lwt.2014.08.028>
- Wallin, A., Giuseppe, D. D., Orsini, N., Akesson, A., Forouhi, N. G., & Wolk, A. (2017). Fish consumption and frying of fish in relation to type 2 diabetes incidence: a prospective cohort study of Swedish men. *European Journal of Nutrition*, 56, 843–852.
- Wandersleben, T., Morales, E., Burgos-Díaz, C., Barahona, T., Labra, E., Rubilar, M., & Salvo-Garrido, H. (2018). Enhancement of functional and nutritional properties of bread using a mix of natural ingredients from novel varieties of flaxseed and lupine. *LWT - Food Science and Technology*, 91, 48–54. <https://doi.org/10.1016/j.lwt.2018.01.029>
- Wang, R., Liu, P., Cui, B., Kang, X., & Yu, B. (2019). Effects of different treatment methods on properties of potato starch-lauric acid complex and potato starch-based films. *International Journal of Biological Macromolecules*, 124, 34–40. <https://doi.org/10.1016/j.ijbiomac.2018.11.207>
- Wang, S., Wang, J., Yu, J., & Wang, S. (2016). Effect of fatty acids on functional properties of normal wheat and waxy wheat starches: A structural basis. *Food Chemistry*, 190, 285–292. <https://doi.org/10.1016/j.foodchem.2015.05.086>
- Wang, Zheng, M., Yu, J., Wang, S., & Copeland, L. (2017). Insights into the formation and structures of starch-protein-lipid complexes. *Journal of Agricultural and Food Chemistry*, 65(9), 1960–1966. <https://doi.org/10.1021/acs.jafc.6b05772>

- Weichselbaum, E., Coe, S., Buttriss, J., & Stanner, S. (2013). Fish in the diet: A review. *Nutrition Bulletin*, 38(2), 128–177. <https://doi.org/10.1111/nbu.12021>
- Winsome Parnell, & Mackay, S. (2011). *A focus on nutrition: key findings of the 2008/09 New Zealand adult nutrition survey*, 5, 1-360. [https://doi.org/ISBN 978-0-478-37348-6](https://doi.org/ISBN%20978-0-478-37348-6)
- Wojtowicz, A., & Moscicki, L. (2014). Influence of legume type and addition level on quality characteristics, texture and microstructure of enriched precooked pasta. *LWT - Food Science and Technology*, 59(2), 1175–1185. <https://doi.org/10.1016/j.lwt.2014.06.010>
- Wolfe, R. R., Baum, J. I., Starck, C., & Moughan, P. J. (2017). Factors contributing to the selection of dietary protein food sources. *Clinical Nutrition*, 37(1), 130–138. <https://doi.org/10.1016/j.clnu.2017.11.017>
- Wolfe, R. R., Rutherfurd, S. M., Kim, I. Y., & Moughan, P. J. (2016). Protein quality as determined by the digestible indispensable amino acid score: evaluation of factors underlying the calculation. *Nutrition Reviews*, 74(9), 584–599. <https://doi.org/10.1093/nutrit/nuw022>
- World Health Organization (WHO). (2016). Global Report on Diabetes. *Global Reports on Diabetes*, 978, 1–88. [https://doi.org/ISBN 978 92 4 156525 7](https://doi.org/ISBN%20978%2092%204%20156525%207)
- Wu, G. (2013). Functional amino acids in nutrition and health. *Amino Acids*, 45(3), 407–411. <https://doi.org/10.1007/s00726-013-1500-6>
- Wu, G. (2016). Dietary protein intake and human health. *Food and Function*, 7(3), 1251–1265. <https://doi.org/10.1039/c5fo01530h>
- Wu, G., Fanzo, J., Miller, D. D., Pingali, P., Post, M., Steiner, J. L., & Thalacker-Mercer, A. E. (2014). Production and supply of high-quality food protein for human consumption: Sustainability, challenges, and innovations. *Annals of the New York Academy of Sciences*, 1321(1), 1–19. <https://doi.org/10.1111/nyas.12500>
- Wu, G., Wu, Z., Dai, Z., Yang, Y., Wang, W., Liu, C., ... Yin, Y. (2013). Dietary requirements of “nutritionally non-essential amino acids” by animals and humans. *Amino Acids*, 44(4), 1107–1113. <https://doi.org/10.1007/s00726-012-1444-2>
- Wu, S., Feng, B., Li, K., Zhu, X., Liang, S., Liu, X., ... Fan, D. (2012). Fish consumption and colorectal cancer risk in humans: A systematic review and meta-analysis. *American Journal of Medicine*, 125(6), 552–559.

- <https://doi.org/10.1016/j.amjmed.2012.01.022>
- Xun, P., Qin, B., Song, Y., Nakamura, Y., Kurth, T., Yaemsiri, S., ... He, K. (2012). Fish consumption and risk of stroke and its subtypes: Accumulative evidence from a meta-analysis of prospective cohort studies. *European Journal of Clinical Nutrition*, 66(11), 1199–1207. <https://doi.org/10.1038/ejcn.2012.133>
- Ye, J., Hu, X., Luo, S., McClements, D. J., Liang, L., & Liu, C. (2018). Effect of endogenous proteins and lipids on starch digestibility in rice flour. *Food Research International*, 106(1), 404–409. <https://doi.org/10.1016/j.foodres.2018.01.008>
- Yildiz, G., Ding, J., Gaur, S., Andrade, J., Engeseth, N. E., & Feng, H. (2018). Microencapsulation of docosahexaenoic acid (DHA) with four wall materials including pea protein-modified starch complex. *International Journal of Biological Macromolecules*, 114, 935–941. <https://doi.org/10.1016/j.ijbiomac.2018.03.175>
- Yu, Z., Wang, Y. S., Chen, H. H., Li, Q. Q., & Wang, Q. (2018). The gelatinization and retrogradation properties of wheat starch with the addition of stearic acid and sodium alginate. *Food Hydrocolloids*, 81, 77–86. <https://doi.org/10.1016/j.foodhyd.2018.02.041>
- Zabar, S., Lesmes, U., Katz, I., Shimoni, E., & Bianco-Peled, H. (2010). Structural characterization of amylose-long chain fatty acid complexes produced via the acidification method. *Food Hydrocolloids*, 24(4), 347–357. <https://doi.org/10.1016/j.foodhyd.2009.10.015>
- Zakaria, Z. A., Abdul Hisam, E. E., Rofiee, M. S., Norhafizah, M., Somchit, M. N., Teh, L. K., & Salleh, M. Z. (2011). *In vivo* antiulcer activity of the aqueous extract of Bauhinia purpurea leaf. *Journal of Ethnopharmacology*, 137(2), 1047–1054. <https://doi.org/10.1016/j.jep.2011.07.038>
- Zhang, B., Huang, Q., Luo, F. X., & Fu, X. (2012). Structural characterizations and digestibility of debranched high-amylose maize starch complexed with lauric acid. *Food Hydrocolloids*, 28(1), 174–181. <https://doi.org/10.1016/j.foodhyd.2011.12.020>
- Zhang, G., & Hamaker, B. R. (2003). A three component interaction among starch, protein, and free fatty acids revealed by pasting profiles. *Journal of Agricultural and Food Chemistry*, 51(9), 2797–2800. <https://doi.org/10.1021/jf0300341>
- Zhang, G., & Hamaker, B. R. (2004). Starch-free fatty acid complexation in the presence

- of whey protein. *Carbohydrate Polymers*, 55(4), 419–424. <https://doi.org/10.1016/j.carbpol.2003.11.005>
- Zhang, G., Maladen, M., Campanella, O. H., & Hamaker, B. R. (2010). Free fatty acids electronically bridge the self-assembly of a three-component nanocomplex consisting of amylose, protein, and free fatty acids. *Journal of Agricultural and Food Chemistry*, 58(16), 9164–9170. <https://doi.org/10.1021/jf1010319>
- Zhang, Li, Z., Wang, Y., Xue, Y., & Xue, C. (2016). Effects of konjac glucomannan on heat-induced changes of physicochemical and structural properties of surimi gels. *Food Research International*, 83, 152–161. <https://doi.org/10.1016/j.foodres.2016.03.007>
- Zhang, Y., Sui, X., & Huang, D. (2017). Mitigating the *in vitro* enzymatic digestibility of noodles by aqueous extracts of Malay cherry leaves. *Food Chemistry*, 232, 571–578. <https://doi.org/10.1016/j.foodchem.2017.04.032>
- Zhao, J., Jiang, Q., Xu, Y., & Xia, W. (2017). Effect of mixed kojis on physiochemical and sensory properties of rapid-fermented fish sauce made with freshwater fish by-products. *International Journal of Food Science and Technology*, 52(9), 2088–2096. <https://doi.org/10.1111/ijfs.13487>
- Zhao, Y. H., Manthey, F. A., Chang, S. K. ., Hou, H. J., & Yuan, S. H. (2005). Qualities of food quality characteristics of spaghetti as affected by green and yellow pea, lentil, and chickpea flours. *Sensory and Nutritive Quality of Food*, 70(6), 371–376. <https://doi.org/10.1111/j.1365-2621.2005.tb11458.x>
- Zheng, M., Chao, C., Yu, J., Copeland, L., Wang, S., & Wang, S. (2018). Effects of chain length and degree of unsaturation of fatty acids on structure and *in vitro* digestibility of starch-protein-fatty acid complexes. *Journal of Agricultural and Food Chemistry*, 66(8), 1872–1880. <https://doi.org/10.1021/acs.jafc.7b04779>